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Bachelor of Science (Honours) in Mathematical Sciences

**Mathematical modelling of the effects of health interventions on the  
evolution of life history in disease-causing organisms**

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## **i Abstract**

We use mathematical models to explore the evolutionary implications of health interventions affecting age-dependent mortality schedules in two contexts, antihelminthics targeting parasitic nematodes, and programs directed against malaria vectors.

We show that interventions targeting parasitic nematodes can exert selection pressure to either shorten or extend the time to maturity, depending on the details of worm mortality functions with and without the intervention. Interventions may therefore generate selection favouring later-maturing, larger and more fecund worms, rather than inevitably favouring the evolution of smaller, less fecund and hence potentially clinically less damaging worms as previously assumed.

The evolution of insecticide-resistant mosquitoes threatens conventional public health programs targeting malaria vectors. By exploiting the high mortality rates of wild mosquitoes and the delay between malaria infection and infectiousness in mosquito hosts, late-life-acting (LLA) insecticides which kill only older mosquitoes can in principle provide effective transmission control in combination with very low selection for resistance. We develop a novel mathematical model to evaluate the potential of such pesticides and find that theoretical LLAs which affect only mosquitoes above a specific age can offer transmission control comparable with conventional insecticides, combined with very low selection for resistance. Benefits are maximised by generating lower mortality in mosquitoes not infected with malaria, and contacting and killing mosquitoes prior to biting. We also explore the optimum virulence characteristics for a fungal LLA biopesticide, and find that it may offer improved transmission reduction as well as lower selection for resistance when compared to some insecticides in current use. Lastly we use our model to assess a candidate for development as a chemical LLA.

Finally, we explore a disparity between our model results and the conventional wisdom that a rare, recessive resistance allele will not spread. We find that the assumption of discrete, non-overlapping generations is key in this context.

## **ii Acknowledgements**

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Several parts of this thesis gave rise to or contributed to the following publications, copies of which can be found at the end of this document ;

*How will public and animal health interventions drive life history evolution in parasitic nematodes?* Penelope A. Lynch, Uwe Grimm, Andrew F. Read, (2008), *Parasitology* 135, 1599–1611

*How to make evolution-proof insecticides for malaria control* Andrew F. Read, Penelope A. Lynch, Matthew B. Thomas, (2009), *PLoS Biol* 7(4)

*Prospective malaria control using entomopathogenic fungi: comparative evaluation of impact on transmission and selection for resistance.* Penelope A. Lynch, , Uwe Grimm, Matthew Thomas, & Andrew Read, (2012), *Malaria Journal* 11, 383

## v Abbreviations

ADI	Age dependent insecticide
AIB	Average infectious bites per mosquito lifetime
CIKI	Conventional instant-kill insecticide
COR	Cost of resistance
EIR	Entomologic inoculation rate
FCM	Feeding cycle model
IRS	Indoor residual spray
ITN	Insecticide-treated bed net
LLA	Late-life acting
LRS	Lifetime reproductive success
OM	Overlap model
OP	Organophosphorous insecticide
PM	Population model
RAIB	Reduction in average infectious bites
RM	Replacement model
SIM	Size independent model
SDM	Size dependent model
SDMP	Size dependent model for pulsed dose interventions
TDI	Time dependent insecticide

*"Maths is the purest science in that you don't need any test tubes or animal testing to do it. All the other sciences eventually boil down to maths, apart from biology, which boils down to soup."*

Guy Browning, The Guardian, 2004.

## 1 General Introduction

Organisms have a wide range of life histories. For example, the African elephant (*Loxodonta africana*) has an average weight of 4.5 tonnes, a lifespan of 70 years, and females produce an average of one offspring every four to nine years. In contrast, the wood mouse (*Apodemus sylvaticus*) has an average weight of 23g, lives for around 1 year, and females produce up to 4 litters per year, with 5 to 7 young per litter. Life history theory is a branch of evolutionary biology which attempts to explain this diversity by considering life history traits as adaptations, based on their close ties to an organism's fitness. This perspective allows us to predict the direction and rate of change in such traits by considering trade-offs and constraints[1].

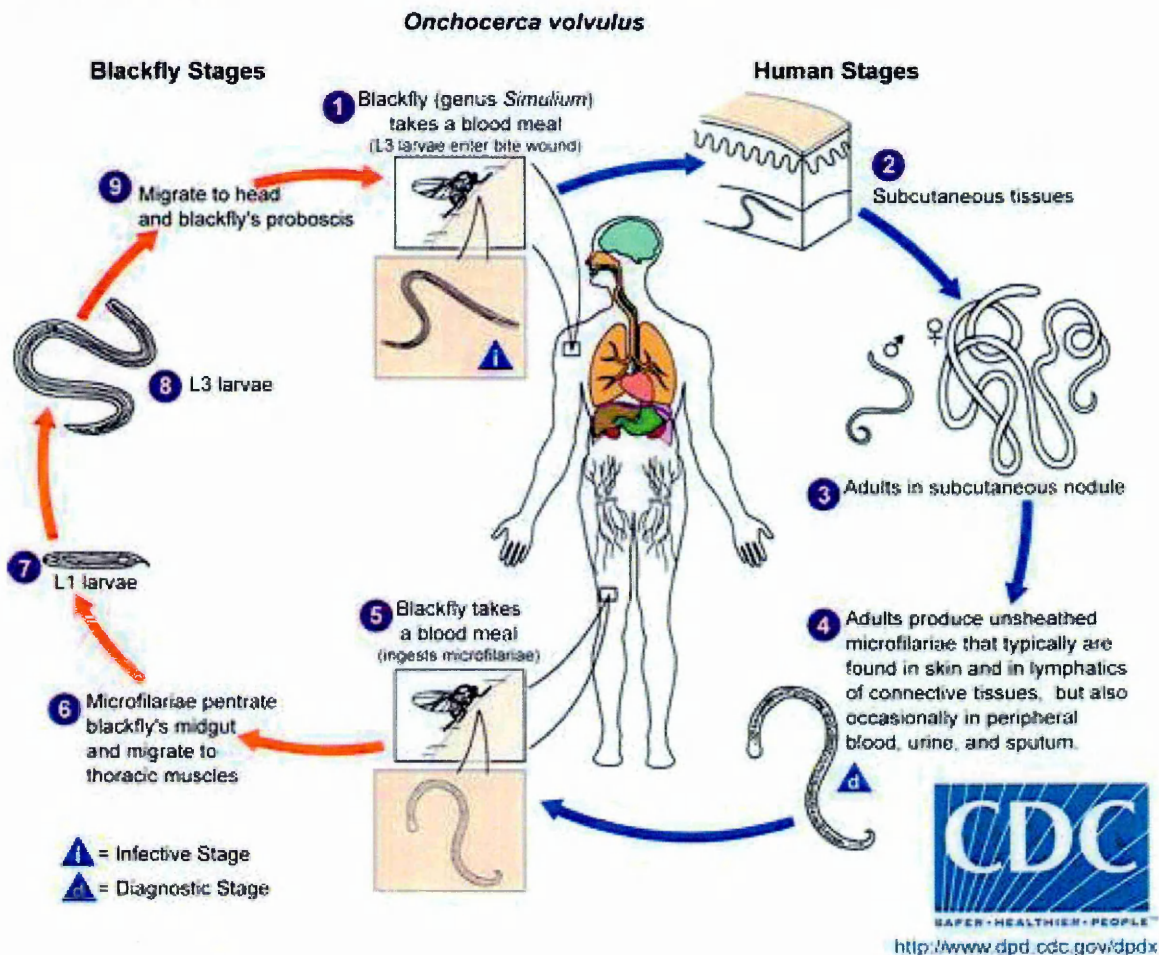
The use of drugs, vaccines, and pesticides to reduce the burden of human disease imposes strong selection on target organisms, as evidenced by the evolution of drug resistance in many microorganisms. Evolutionary biology enables us to predict the direction and rates of change in response to such man-made selection. Here we are concerned with the evolutionary consequences of age-dependent mortality, a key driver of the evolution of many traits. We consider its effects on age at maturity in parasitic worms, and insecticide resistance in mosquito vectors of malaria, with the purpose of understanding likely evolutionary responses and hence informing evolutionary management strategies in an effort to avoid undesirable outcomes.

Parasitic worms are a continuing source of morbidity and mortality, in humans and domestic animals across the globe, causing great suffering and generating a high economic cost. Human diseases caused by parasitic worm infections include onchocerciasis or "river blindness", caused by infection with "*Onchocerca volvulus*" (see Figure 1), the second most important cause of infectious blindness, with approximately thirty-seven million

people infected worldwide [2]. *Ascaris lumbricoides*, the commonest helminth parasite of humans, grows to approximately 30cm, causes a range of symptoms, including direct intestinal blockage, and is estimated to infect around one billion people.

**Figure 1 Lifecycle of *Onchocerca volvulus***

Life cycle of *Onchocerca volvulus*, a parasitic worm which causes Onchocerciasis in infected human hosts. Transmission between infected humans is via an intermediate blackfly host, which become infected with microfilariae when feeding on an infected human, and generates a new infection, after a period of development by the parasite, by transmitting parasite larvae during a subsequent feed on another human host.



In livestock, the annual cost of infection with gastro intestinal parasites, including prophylaxis, treatment and lost performance is estimated at £84 million per annum for the UK sheep industry alone [3].

In response to these damaging infections, a wide range of antihelminthic interventions have been developed and applied, including drug treatments for infected individuals, and prophylaxis for human and veterinary use. Antihelminthics are routinely used to maintain the health and productivity of livestock [4], and multiple large-scale public health

interventions have been initiated, designed to alleviate or eliminate human disease arising from infection with parasitic worms [2]. These include programs which specifically target the elimination of specific parasites, not merely the prevention of human disease caused by parasites. GPELF, the Global Program to Eliminate Lymphatic Filariasis and OEPA, the Onchocerciasis Elimination Program for the Americas are examples of such campaigns and are providing regular doses of antihelminthic drugs on a huge scale, more than half a billion people have received treatment as part of the program spearheaded by GPELF [5]. Such programs inherently generate changes to parasite mortality schedules, a key life-history determinant. We consider in chapter 2 one possible evolutionary outcome of such alterations to parasite mortality, a change in the age at which worms reach maturity and begin to reproduce. Previous theory indicated that selection generated by increased mortality rates would always favour earlier maturity and hence smaller, less fecund worms [6]. Using a new mathematical model which generalises the existing models by taking into account the effects of age-related mortality we find, however, that if adult mortality rates vary with age at maturity, interventions which change mortality schedules could generate selection favouring later maturing, and hence larger and more fecund worms.

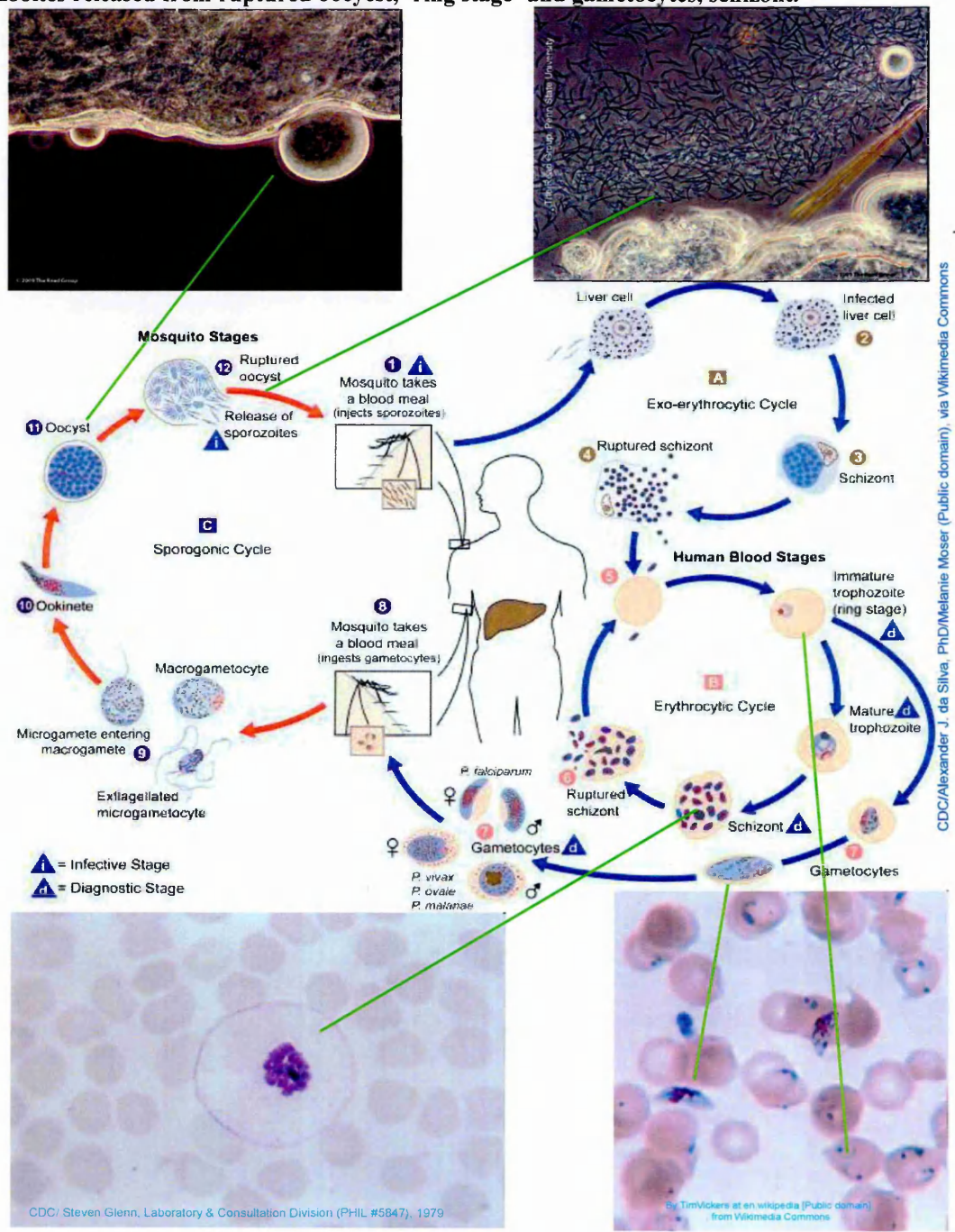
The importance of age-related mortality also informs the concept of late-life acting insecticides for use in the control of malaria transmission. Globally malaria continues to be a major cause of mortality and morbidity [7], despite a long history of public health campaigns, and successful eradication in many formerly endemic areas [8]. The malaria parasites which cause human disease, primarily *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale* [9], all have life histories which requires alternation between human and mosquito hosts (Figure 2). Although the various *Anopheles* mosquito species which can host *Plasmodium* are in fact the primary host, since sexual reproduction occurs during *Plasmodium*'s development in the mosquito, our naturally anthropocentric view of the transmission process leads to definition of the



mosquito host as a malaria ‘vector’, the means by which the disease is carried between humans. Action to combat malaria thus has two potential modes of action, combating the parasite directly, or targeting the means by which it is spread, the mosquito vector.

**Figure 2 Life history of *Plasmodium* parasite**

Following infection via an infected mosquito bite, the *plasmodium* parasite passes through a series of developmental stages in the human host. Onward transmission to a mosquito host can only occur when gametocytes are present in the bloodstream. A mosquito from a suitable vector species which ingests gametocytes may become infected, *Plasmodium* progresses through the ookinete, oocyst and sporozoite stages in the mosquito host over a number of days. Once sporozoites are present in the salivary glands, *Plasmodium* can be transmitted to a human host when the mosquito vector takes another human blood meal. Photographic images, clockwise from top left; oocyst on mosquito midgut, sporozoites released from ruptured oocyst, ‘ring stage’ and gametocytes, schizont.

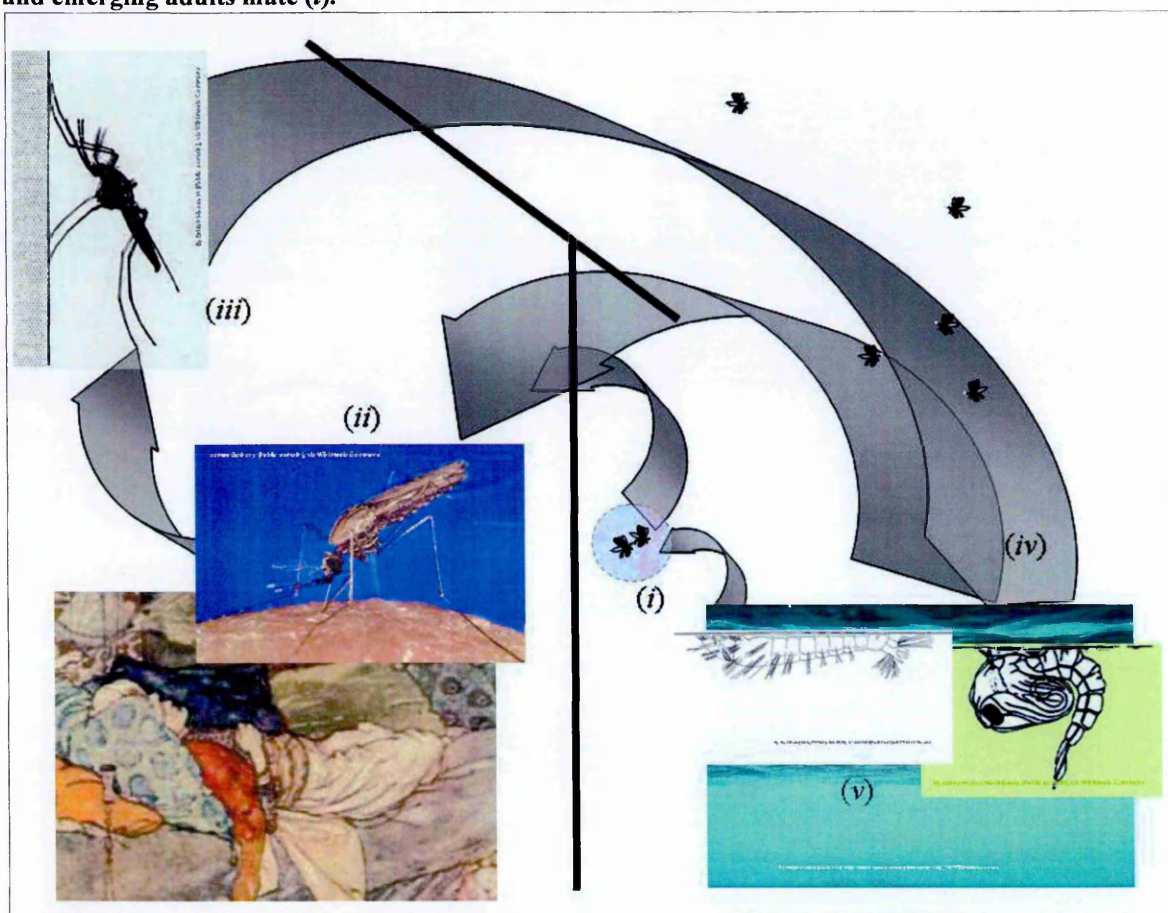




For vector-targeted malaria control, the biology of the *Anopheles* mosquito hosts is key to defining effective strategies. Mosquito life history follows a cyclical pattern of blood feeding, resting, egg-laying, host seeking, feeding again, and so on (Figure 3). For most malaria vectors blood feeding takes place at night, inside human habitations.

### Figure 3 Generalised *Anopheles* lifecycle

Lifecycle of malaria vector (i) mating, (ii) blood feed on human host, (iii) rest, commonly on interior surface, (iv) find suitable laying site and oviposit, (v) eggs hatch, larva grow and pupate, pupae mature and emerging adults mate (i).

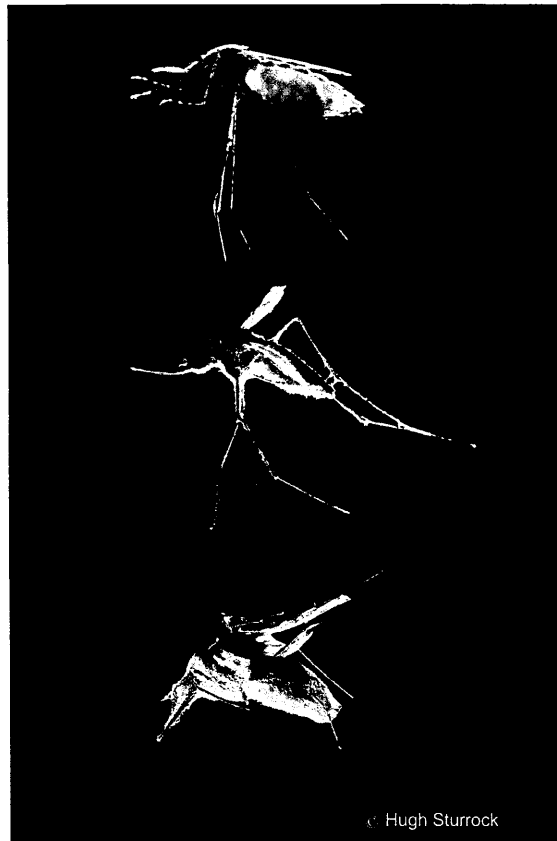


Where the physical environment and the specific biology of local vector species permit, removal of mosquito breeding sites, for example through drainage schemes, has proved a lasting and effective means of reducing mosquito numbers and hence malaria transmission. Where such measures are impractical or uneconomic, however, the primary means of controlling adult mosquitoes is through the use of chemical insecticides, sprayed on the interior walls where mosquitoes rest after a blood meal [10] (IRS), or applied to bed nets (ITNS). Following long and successful use of these methods, increasing incidences of

vector populations resistant to commonly used insecticides threaten the long-term efficacy of insecticide based programs [11-15].

There is therefore a need for alternative control methods which avoid the repeated cycle of developing new products to replace those lost to resistance, followed in due course by the loss of the new compounds as resistance to them also spreads. In chapter 3 we evaluate one such alternative, late-life acting (LLA) insecticides which aim to achieve effective malaria transmission control in combination with minimal selection for resistance, by selectively changing age-linked mortality profiles for malaria vectors. The high mortality of wild mosquitoes and the relatively long period of development required before a *Plasmodium* infection in a mosquito host is transmissible, mean that older mosquitoes generate few eggs but are responsible for all infectious bites. Interventions which target only old mosquitoes, therefore, could potentially have little effect on the relative fitness of susceptible mosquitoes, few of whom will live long enough to be affected, whilst eliminating the majority of infectious bites. To explore whether the ideal of good performance for both criteria is theoretically possible requires a new modelling framework which captures mosquito survival, blood feeding and reproduction probabilities in a detailed, age-structured format. We therefore developed a new, two part mathematical model, the first part of which is a deterministic, markovian, feeding cycle based model, which tracks the key probabilities of age-dependent survival, infection, and reproduction for mosquitoes subject to a range of interventions. The results from the feeding cycle model provide data for the second part of our model, a population model which calculates the speed of spread of resistance through a population comprising a mixture of resistant and susceptible genotypes. We confirm that age-linked insecticides, killing on contact much like existing insecticides, but only affecting mosquitoes above a defined age, could meet the aims of the LLA concept, and provide transmission control without strong selection for resistance. We also consider the contexts in which the benefits of such products would be maximised.

**Figure 4** *Anopheles stephensi* following infection with *Beauveria bassiana* fungus. *Anopheles stephensi*, freshly fed (top), and killed by *Beauveria bassiana* infection 24 hours (middle) or 40 hours (bottom) previously, showing sporulation. Image provided by the Read Group, Penn State University.



Fungal biopesticides already used commercially are under development for use against malaria vectors (Figure 4), and their natural development pattern in infected mosquitoes, a period of growth prior to the onset of increased mortality, [16-18], makes them candidate LLA biopesticides. Multiple strains are potentially available, offering a wide range of virulence characteristics in *Anopheles*. In chapter 4 we characterise fungal virulence using a simple, two-parameter function, which we use with the LLA models developed in chapter 3 to determine the virulence characteristics which offer the best combinations of resistance management and disease control, providing guidance for current and future processes of strain selection or modification. We also compare the potential performance of fungal biopesticides with that of existing products, and find that, for IRS, they may offer better transmission control than some widely used conventional insecticides which, unlike

fungal spore preparations [19], have high contact irritancy, and repel a proportion of mosquitoes before they can receive a fatal dose [20].

Serpins are biologically active protein molecules, found in all higher eukaryotes, as well as bacteria and viruses. In insects they control aspects of the immune system, including melanisation, and Serpin-2 (SRPN2) regulates melanisation in mosquitoes [21], such that melanisation is upregulated when SRPN2 is depleted. This process produces age-related changes to the mosquito mortality schedule, and a chemical LLA able to produce the same effect as SRPN2 depletion therefore presents as a potential target for development as an LLA insecticide. We were approached by the researchers working on SRPN2 and asked to assess the LLA potential of such a product, based on experimental data generated using SRPN2 depleted mosquitoes (SRPN2KD). In addition to mortality data, the experimental results provided information on reduced feeding propensity and fecundity in the SRPN2KD mosquitoes. In chapter 5 we explain how we processed the experimental data to provide mortality, fecundity and feeding propensity values to parameterise an amended version of our LLA model. Our analysis shows that the transmission reductions achievable by a product producing SRPN2 depletion are highly dependent on reduced feeding propensity. Since mosquitoes which have not blood fed do not produce eggs, reduced feeding propensity also directly increases the relative fitness of resistant mosquitoes, so that performance with respect to one criterion for a successful LLA insecticide, transmission reduction, comes at a direct cost to performance regarding the other criterion, low selection for resistance.

Finally we establish the importance of detailed life history parameters by considering an area of modelling where they are commonly overlooked. In analyses designed to predict the speed of spread of resistance alleles in a population, the conventional wisdom is that whilst a dominant resistance allele will spread, and the rate of spread will be substantially affected by the relative fitness of susceptible and resistant phenotypes, the speed of spread

of a single-locus, rare, recessive resistance allele will be negligible, and the effect of relative fitness on the speed of spread will not be material. This assumption informs many practical resistance management strategies. The standard population genetics model which underpins this idea is predicated on a number of assumptions, including the premise that generations are discrete, the population comprising a sequential series of cohorts which mature, reproduce for a period and die, to be replaced by their offspring. Whilst this assumption is valid for many pest life-histories, it is not true for populations like *Anopheles* mosquitoes, in which the breeding population comprises a mixture of newly mature adults and surviving older adults of various ages, all of whom contribute to the allele proportions in the next generation of offspring. We show numerically that the assumption of discrete generations is key to the calculated rate of spread when using the standard model to predict the spread of initially rare, recessive resistance alleles. If the standard model is amended to incorporate adult survival between periods the calculated rate of spread of rare recessive alleles increases, it increases more when greater overlap is assumed, and the relative fitness benefits of resistance become increasingly material with increasing overlap. These results are consistent with the results generated using our detailed, age-structured mosquito population model. Although mathematical comparison of the rates of change with and without adult survivorship between generations is rendered somewhat intractable by the non-linear, iterative nature of population genetics calculations, in addition to our numeric analysis, we have also been able to show mathematically that including overlap will always generate a more rapid rate of growth in the proportion of resistance alleles in the population if given initial conditions are met.

The evolution of pathogens and disease-vectors in response to health interventions is occurring so fast that we can observe it happening; multiple drug resistant bacteria and insecticide resistant vectors emphatically remind us that evolution is not merely history, or theory. It has huge, immediate, practical implications, and work to understand, anticipate, and manage this process has enormous potential benefits. Although the complexity of the

living world at every level presents a challenge to the modelling process, the essential trade-offs at the heart of evolutionary biology are ideal subjects for mathematical modelling, and in the following chapters we develop models to answer questions about how efforts to combat disease may drive evolution in target organisms, and whether we can reduce the probability of undesirable evolutionary outcomes without compromising our aims in disease control.

The author has chosen to lift the quality of the prose in this document by including a few quotations from great minds of the past. She has additionally chosen to enliven the text with one bad limerick of her own. This draws on a long and well established tradition of bad verse in science [22-24], and should be considered above reproach.

*"If the people do not believe that mathematics is simple, it is only because they do not realize how complicated life is"*

(J. von Neumann)

## **2 Chapter 2 How might public and animal health interventions drive evolution of age at maturity in parasitic nematodes?**

### **2.1 Introduction**

Infections by parasitic nematodes have a large impact on the health of humans and domestic livestock. Two key life-history traits, fecundity and body size, are important determinants of nematode infectiousness and host damage [25,26]. Both are dependent on the age at which nematodes mature, since nematodes stop growing at or around maturity and begin to reproduce, and since fecundity is correlated to adult body size. A longer growth period permits greater size and larger worms can produce more eggs [6,25,27-29]. Conversely, a shorter growth period reduces the probability of dying pre-maturity and hence failing to reproduce at all. This trade-off means that age at maturity must be subject to intense natural selection. Here we ask how widespread health interventions such as vaccination and chemotherapy campaigns, which change mortality rates and hence affect this trade-off, might alter nematode life history evolution. Most previous work has shown that smaller, less fecund worms are the likely outcome [6,30, but see 31,32]. In this chapter we show that a variety of evolutionary outcomes are possible, some of which are likely to result in the evolution of larger and hence more fecund and damaging worms.

Previous theoretical work on the evolution of parasitic nematode life histories has followed standard life history theory [33,34] and assumed that mortality schedules are the major determinants of selection [6,25,29,35,36]. Where chances of survival are high, nematodes should delay maturity to gain the fecundity benefits of large size. However, when chances of survival are low, worms should mature early in order to achieve some reproduction before death, even if this means they mature at small size and hence have low fecundity.

Thus, where daily survival rates are high, one might expect a life history like that of *Ascaris lumbricoides*, for example, which reaches up to 30cm in length and produces 25 million eggs over a lifetime. In contrast, where chances of survival are low, natural selection should favour a life history like that of the pin worm, *Enterobius vermicularis*, which has a maximum length of 1 cm and produces no more than 10,000 eggs. A formal model of this idea, together with experimental data on survival rates, explains about 50 percent of the cross-species variation in age to maturity of parasitic nematodes of mammals [6].

The aim of animal and human health programmes like chemotherapy and vaccination is to increase worm mortality. Thus, nematode life histories could evolve in response to such interventions [28,30-32,37]. This evolution may in principle occur in parallel with, or instead of, the evolution of drug- or vaccine- resistance. There is no direct evidence yet of such evolution, but it has not to our knowledge been looked for [for indirect evidence, see 28]. Where it has been looked for, in other contexts, life history evolution in response to anthropogenic alterations in mortality schedules has been demonstrated. For instance, changing size-dependent mortality schedules by size-selective harvesting of populations of Atlantic silverside (*Menidia menidia*) produced rapid evolution of slow growing, smaller fish in large-harvested populations and fast-growing, larger fish in small-harvested populations [38]. Rapid evolution of life-history traits in response to non-anthropogenic environmental change has been observed in *Rhabdias pseudosphaerocephala* [39] at the range-edges of its toad host as it expands its geographic range.

Most previous theoretical work on the evolution of nematode age in response to medical and veterinary intervention has suggested that the resulting life history evolution would be beneficial from a disease control stand point. The argument is that intervention-induced increases in mortality will mean that natural selection will always favour earlier maturation and thus result in smaller and less fecund worms [6,30,32]. However, existing formal



models of this make restrictive assumptions about the nature of nematode mortality patterns, in particular assuming that mortality rates are unaffected by age at maturity. Here we formally analyse earlier verbal suggestions [6,31,37] that some types of stage- or size-specific mortality might generate clinically-detrimental life history evolution.

It seems highly likely that mortality rates will vary with worm size. All else being equal, larger nematodes presumably provide more stimulus to the immune system, because they will secrete more antigens and have a larger surface area, and may do more damage. Alternatively, smaller nematodes may be more vulnerable to immune attack if they are less able to withstand damage from a given number of effector molecules. Immunity can also differentially affect the survival of different developmental stages of parasites. For example, in *Strongyloides ratti* different mortality rates are observed for larval and adult stages which are in different host tissues [40]. The host immune response can also alter worm fecundity directly and indirectly via its effects on worm size [41,42]. Here we consider the effects of chemotherapy and vaccination allowing for these sort of more complex mortality schedules. We also consider the effects both of changes in mortality schedules which might be continuous (eg. vaccination or, in the case of farm animals, artificially-selected resistant hosts) or those which would be pulsed (e.g. many chemotherapeutic regimes used in an agricultural context). We show that optimism emerging from previous models maybe misplaced: in some circumstances, health interventions may select for increased time to maturity, which would result in larger and more fecund worms.

## 2.2 Models

We consider two types of intervention. The first is where the entire natural lifespan of the worms can be expected to fall within a period where the intervention is having an effect, as would be the case for immunisation or enhanced resistance by selective breeding; for simplicity we consider this under the general heading of 'sustained interventions'. The

second is where the intervention acts as series of brief, regularly spaced, discrete events against the background of the underlying mortality rates, as commonly occurs with chemotherapy in an agricultural context, where animals are routinely drenched at particular intervals. We refer to this as 'pulsed interventions'. These two situations need to be modelled in different ways, so we consider each in turn.

All models assume that worm births are steady over time and the population is in equilibrium, hence lifetime reproductive success (measured as lifetime egg production) is an appropriate measure of fitness. Anderson and May [43] provide evidence supporting this assumption. Analysis of the epidemic situation, where other fitness measures are more appropriate, is beyond the scope of this study.

### 2.3 Size-independent mortality model for sustained interventions

Here we consider the size-independent mortality model (henceforward "SIM" model) developed by Gemmil *et al.* [6], and introduce our new model, which incorporates size-dependent mortality (henceforward "SDM" model). We then use these models to study the effect of public and animal health interventions on worm life history evolution.

Throughout, symbols are as given in Table 1, and all mortality rates are instantaneous rates – the probability of death at any particular point in time.

The assumptions of the SIM are as follows [6]

1. Worms grow throughout development, but growth ceases at maturity.
2. Per unit time fecundity increases with worm size and hence with maturation time  $\alpha$ , according to the relationship  $\text{fecundity} = c\alpha^\beta$ .
3. Within the host, parasites experience a constant juvenile mortality rate,  $M_j$ , until maturation.
4. After the onset of reproduction, parasites experience a constant adult mortality rate,  $M_a$ .

<b>Table 1: Variables and Parameters for SIM, SDM and SDMP models.</b> <b>Note all ages are measured from first infection of the mammalian host.</b>	
$\alpha$	age at maturity
$\omega(\alpha)$	fitness of worms maturing at $\alpha$
$c$	constant relating age at maturity to worm fecundity
$\beta$	exponent of allometric relationship relating age at maturity to fecundity
$M_j$	within-host mortality rate for juvenile parasites
$M_a$	within-host mortality rate for adult parasites
$m(z)$	mortality rate experienced by juvenile parasites at age $z$
$d(\alpha)$	mortality rate experienced by adult parasites which matured at age $\alpha$
$\omega_h(\alpha)$	fitness of worms maturing at $\alpha$ in hosts experiencing a health intervention
$\beta_h$	allometric exponent relating fecundity to age at maturity in hosts experiencing a health intervention acting to reduce rate of increase of fecundity with age
$m_h(z)$	mortality rate experienced by juvenile parasites at age $z$ in hosts experiencing a health intervention acting to increase juvenile parasite mortality
$d_h(\alpha)$	mortality rate experienced by adult parasites which matured at age $\alpha$ in hosts experiencing a health intervention acting to increase adult parasite mortality
$s_h(\alpha^*)$	Selection gradient at $\alpha^*$ under an intervention

The probability of survival to maturation at time  $\alpha$  is derived by treating the occurrence of death as a random variable with distribution Poisson( $\lambda$ ) where  $\lambda$  is the mortality rate,  $M_j$ . Thus, the average lifetime fecundity for individuals maturing at  $\alpha$  is given by

$$\omega = c\alpha^\beta e^{-M_j\alpha} \frac{1}{M_a} \tag{1}$$

The model comprises three elements:  $c\alpha^\beta$ , the daily fecundity following maturity at  $\alpha$ ;  $e^{-M_j\alpha}$ , the probability of survival to maturity with pre-patent period  $\alpha$ ; and  $\frac{1}{M_a}$ , the life expectancy post-maturity (assuming survival times are exponentially distributed).

The age at maturity favoured by natural selection,  $\alpha^*$ , corresponds to the maximum of  $\omega(\alpha)$ , at which the derivative  $\omega'(\alpha^*) = 0$ , namely

$$\alpha^* = \frac{\beta}{M_j} \tag{2}$$

The same result can be derived from an explicitly epidemiological framework. Morand and Poulin [36] derived an alternative model for the relationship between parasite mortality

rate and optimal time to maturity using  $R_0$ , the basic reproductive rate, based on explicit epidemiology, as follows;

$$R_0 = \frac{(\alpha^a)^c \beta H}{\alpha (\mu_w + \beta H) \left( \frac{1}{\alpha} + b + \mu_L \right) (b + \mu_p)} \quad (3)$$

giving

$$\alpha^* = \frac{-ca}{(ca-1)(\mu_L + b)} \quad (4)$$

with symbols as in Table 2, equation (4) differs from equation (2). However, we show here that the two models give an equivalent solution for optimal age to maturity.

The derivation of equation (3) is based on a model by Anderson and May [43],

$$R_0 = \frac{ks\Phi\beta d_1 d_2 N \lambda}{(\mu + \mu_1)(\mu_2 + \beta N)}$$

which separates the parasite mortality rate into two components, mortality of parasites within a living host, and parasite mortality through host death. The Anderson and May model also reflects a period of larval development outside the host prior to infectiousness, and a subsequent period of viability in the environment during which infective larvae may contact and infect hosts. Morand and Poulin [36] ignore aggregation and implicitly assume that all worms are hermaphrodite, so the parameters  $k$ ,  $s$ , and  $\Phi$  in the Anderson and May model can be ignored.

Morand and Poulin [36] give the proportion of larvae infecting hosts which ultimately become adults within the host as  $\frac{1}{\alpha \left( \mu_L + b + \frac{1}{\alpha} \right)}$ . This seems to be replicating the

Anderson and May formula for the proportion of eggs produced which ultimately infect hosts, given by the probability of survival to infective stage  $\times$  life expectancy of infective larvae in the environment  $\times$  per day transmission rate. However, this is not an appropriate representation of the process of in-host maturity where the transition from juvenile to adult

occurs at age  $\alpha$  for all larvae surviving to age  $\alpha$ , not randomly at a given rate after age  $\alpha$  has been reached. In addition, the use of  $1/\alpha$  as the rate at which immature parasites become mature is inappropriate, since maturation does not happen randomly across all ages of immature parasites, but only to the proportion which have survived to age  $\alpha$ , and this would only be  $1/\alpha$  in the case where the in-host mortality rate among immature parasites was zero.

**Table 2** Equivalence of parameters used in three models, ‘A and M’, from Anderson and May [43], ‘M and P’, from Morand and Poulin [36] and SIM, the model of Gemmill et al. [6] .

A and M	Parameter Description	M and P	SIM
$k$	parameter summarising aggregation of parasites within host population	not explicitly included	n/a
$s$	proportion of females in parasite population	not explicitly included	n/a
$\Phi$	mating function	not explicitly included	n/a
$\beta$	transmission co-efficient between host and infective stages	$\beta$	n/a
$d_1$	proportion of parasites entering host which survive to maturity	not explicitly included	n/a
$d_2$	proportion of output transmission stages surviving to infective stage	assumed immediately infective	n/a
$N$	host density	$H$	n/a
$\mu$	in-host parasite mortality rate arising from host death	$b$	part of $M_j$ and $M_a$
$\mu_1$	in-host parasite mortality rate arising from other causes	immature $\mu_L$ mature $\mu_p$	part of $M_j$ part of $M_a$
$\mu_2$	free-living parasite mortality rate	$\mu_w$	n/a
$\lambda$	fecundity / eggs per day	$\lambda = \alpha^{ac}$	$c\alpha^\beta$

Using the parameters of the Morand and Poulin model, the corrected formula for the proportion of immature parasites which survive a period of  $\alpha$  days from arrival in-host to reach maturity is  $e^{-(\mu_L+b)\alpha}$

Incorporating this means that equation (3) becomes

$$R_0 = \frac{\alpha^{ac} \beta H}{(\mu_w + \beta H)(b + \mu_p)} e^{-(b+\mu_L)\alpha}$$

(5)

giving

$$\alpha^* = \frac{ca}{(b + \mu_L)}$$

(6)

Since  $(\mu_L + b)$  is the total mortality rate for immature parasites, equivalent to  $M_j$  in the SIM model, and  $ca$  is equivalent to  $\beta$  in the SIM model, equations (6) and (2) are equivalent.

## 2.4 Size-dependent Mortality Model for sustained interventions

We now extend the size-independent model (Equation 1) to include size-dependent mortality before and after maturation.

To incorporate size-dependent mortality, we replace assumptions (3) and (4) above with the following

5. Pre-maturity mortality rate is determined by size, and so changes during larval development. It is given by the function  $m(z)$ , where  $z$  is the time (age) from arrival in host.
6. Adult parasites experience constant mortality, determined by the size at which they matured, and given by the function  $d(\alpha)$ .

Since the size-dependent mortality model (SDM) has a mortality rate which varies with time, the occurrence of death is a non-homogeneous Poisson process with distribution  $\text{Poisson}(m(z))$ . Thus, the probability that death will not occur before age  $z$  is given by

$$1 - F(z) = e^{-\mu(z)}$$

where

$$\mu(z) = \int_0^z m(u) du \quad (z > 0)$$

Fitness is therefore given by

$$\omega(\alpha) = c\alpha^\beta e^{-\mu(\alpha)} \frac{1}{d(\alpha)} \quad (7)$$

which reduces to  $\omega = c\alpha^\beta e^{-M_j\alpha} \frac{1}{M_a}$ , equation (1), for constant mortality rates  $m(z)=M_j$

and  $d(\alpha) = M_a$ .

The optimal value,  $\alpha^*$ , is again determined by the condition  $\omega'(\alpha^*) = 0$ . Thus,

$$0 = \frac{\beta}{\alpha^*} - \frac{d'(\alpha^*)}{d(\alpha^*)} - m(\alpha^*) \quad (8)$$

with the additional requirement that, to ensure  $\omega(\alpha)$  is maximal at  $\alpha = \alpha^*$ , the second derivative must be negative.

As illustrated in Figure 8, multiple solutions may be possible for some combinations of mortality functions so that the theoretical global optimum may not always be the value selected for.

## 2.5 The effects of sustained interventions on optimum time to maturity

With size-dependent mortality, there is no generalised equation for  $\alpha^*$  analogous to equation (2). However, an indication of the immediate direction of selection on age to maturity under an intervention can be determined by the sign of the selection gradient, the derivative of the fitness function under the intervention, in the vicinity of the pre-intervention value of  $\alpha^*$ . This corresponds to the sign of  $s_h(\alpha^*)$  where

$$s_h(\alpha^*) = \frac{\beta_h}{\alpha^*} - \frac{d'_h(\alpha^*)}{d_h(\alpha^*)} - m_h(\alpha^*) \quad (9)$$

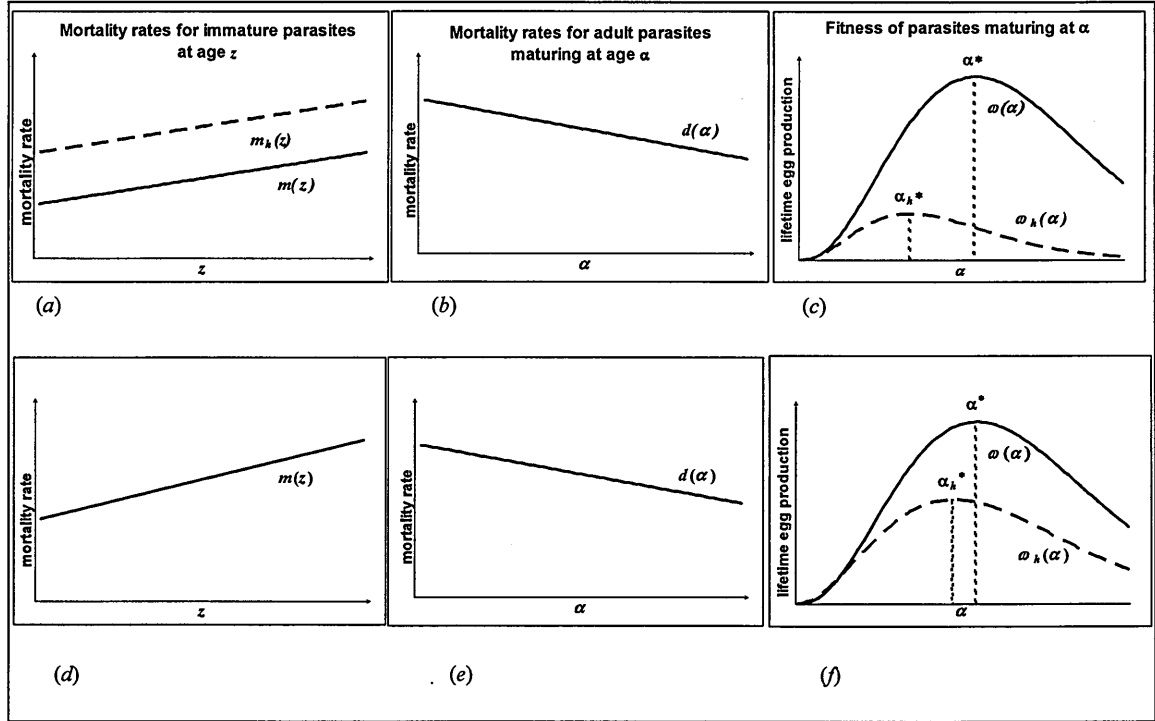
with one or more of  $\beta_h$ ,  $d_h(\alpha^*)$  and  $m_h(\alpha^*)$  affected by an intervention. When equation (9) is positive, the intervention is creating selection pressures that favour worms which grow for longer before reproduction; when equation (9) is negative, natural selection favours shorter maturation periods. Note that this selection gradient approach applies only in the immediate region of the pre-intervention  $\alpha^*$ . Where multiple solutions are possible the overall direction of evolutionary change may be different.

Inspection of equation (9) reveals the following. All else being equal, a health intervention which changes the pre-maturity mortality function to  $m_h(z)$ , with greater mortality for a given size ( $m_h(z) > m(z)$ , for all relevant values of  $z$ ) will always favour reduced time to maturity. This is also true for size-independent mortality (equation (2);[6]). In both cases, this is because greater prematurational mortality selects for earlier reproduction, despite the

fecundity costs, to ensure that worms survive to reproduce at all. Similarly, an intervention which changes the rate of increase of fecundity with size, so that worms are less fecund for a given size (i.e.  $\beta$  to  $\beta_h$  such that  $\beta_h < \beta$ ), will make  $s_h(\alpha^*) < 0$ , so that initial selection pressure will always favour a reduced time to maturity. This too is true for size independent mortality (equation (2); [6]), and is because the intervention is reducing the fecundity gains which accrue through delayed reproduction. Thus, interventions which increase juvenile mortality or decrease the rate of increase of fecundity with worm size will favour the evolution of an earlier age at maturity which will result in smaller and less fecund worms, whether or not mortality rates are size-dependent. These effects are illustrated in Figure 5.

**Figure 5: Illustration of the effects of interventions which increase juvenile mortality or reduce fecundity.**

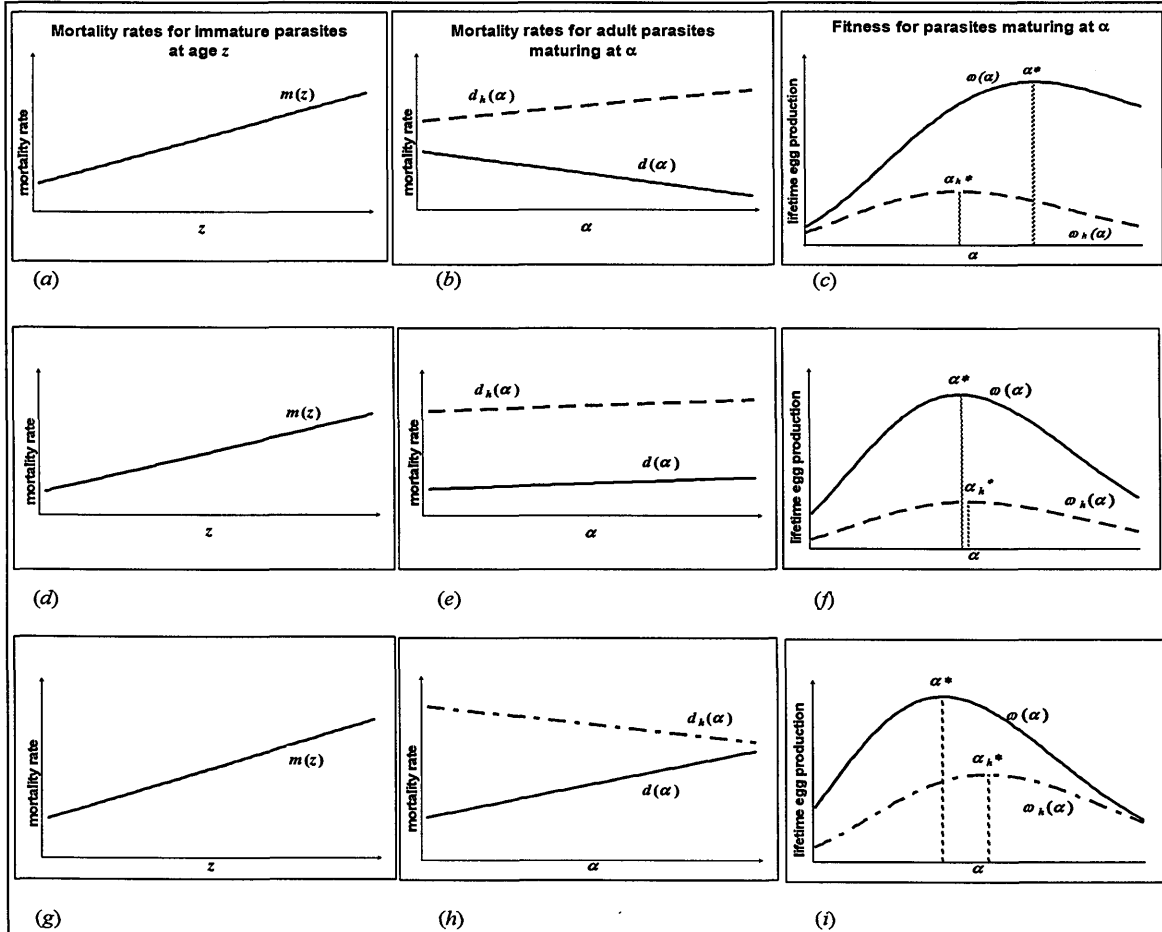
Panels (a) to (c) illustrate the effects on fitness of an intervention which increases the juvenile mortality rate from  $m(z)$  to  $m_h(z)$ , and panels (d) to (f) show the effect of an intervention which leaves the mortality rates unchanged but reduces the rate at which fecundity increases with age at maturity. In both cases the fitness function under the intervention reaches its maximum with a shorter time to maturity ( $\alpha_h^*$ ) than that without the intervention ( $\alpha^*$ ). Continuous lines show functions without the intervention, dashed lines with the intervention.





**Figure 6: Illustration of the effects of interventions increasing the adult mortality rate for parasites maturing at age  $\alpha$ .**

Panels (a) to (c) show an intervention which increases the proportionate rate at which adult mortality rate changes with age at maturity, resulting in a reduction in optimum time to maturity. Panels (d) to (f) show an intervention which keeps the same rate of increase in mortality rate, so that, with higher absolute mortality, there is a reduced proportionate rate of increase and hence an increased optimum time to maturity. Panels (g) to (i) show an intervention with reduced rate of increase in mortality rate, and also reduced proportionate rate of increase in mortality, as might result if an intervention more easily resisted by larger worms outweighed the effects of an immune response more easily evaded by smaller worms, giving an increased optimum time to maturity. Continuous lines show functions without the intervention, and dashed lines with the intervention.



An intervention which affects mortality rates of mature worms has more complex effects on the optimal age to maturity. Inspection of equation (9) shows that the direction of

selection under the intervention depends upon the difference between  $\frac{d'(\alpha^*)}{d(\alpha^*)}$  and

$\frac{d'_h(\alpha^*)}{d_h(\alpha^*)}$ , the proportionate rates of change in mortality with size before and after imposing

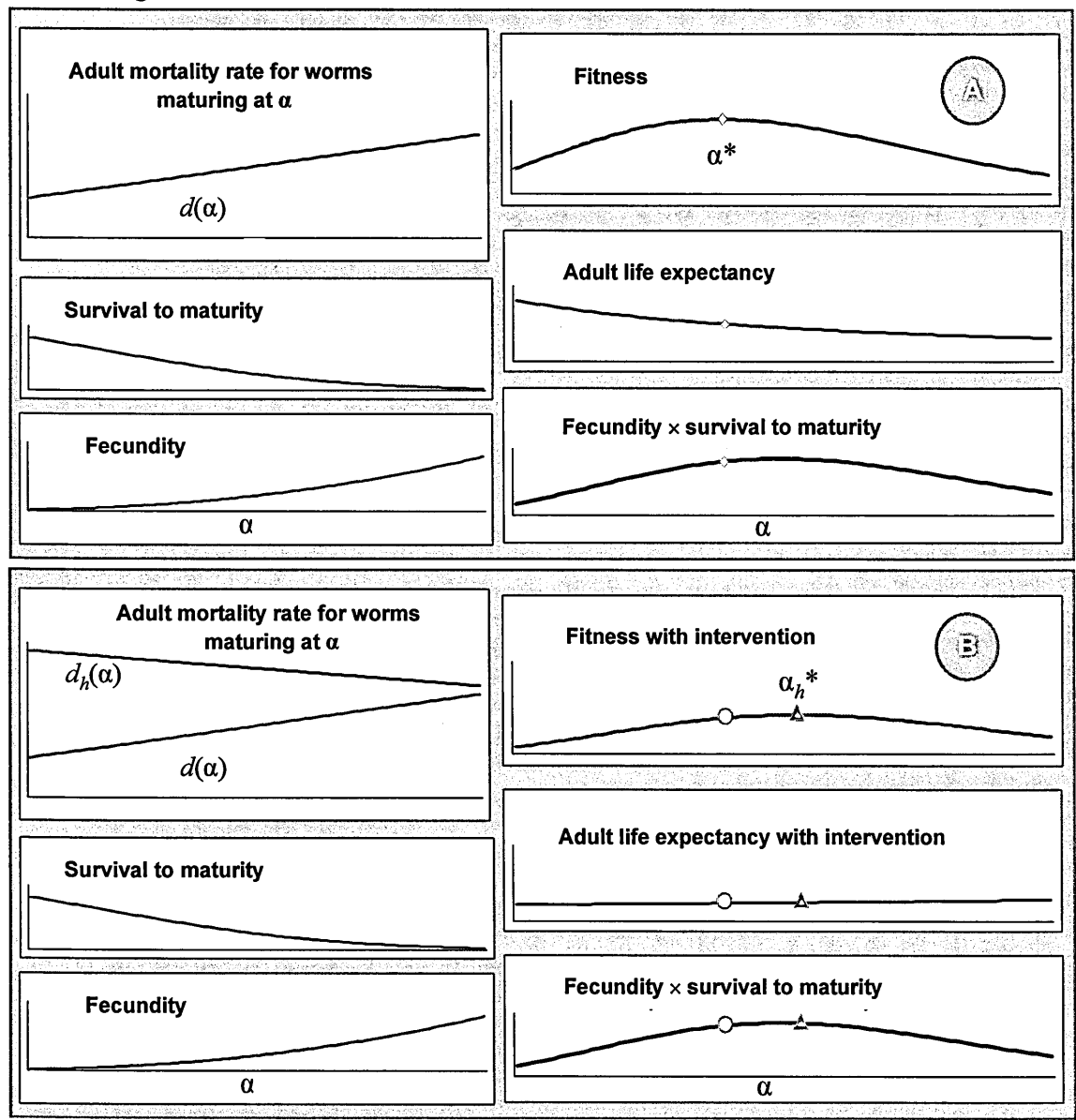
the intervention. This difference depends in turn upon the detail of each function around  $\alpha^*$ . If the difference is positive, then the initial selection pressure under an intervention will favour earlier maturing worms (Fig. 6 a-c). If the difference is negative, as is always

the case if the slope of  $d_h(\alpha)$  is less than or equal to that of  $d(\alpha)$ , then interventions to increase adult mortality will always favour worms which delay maturation (Figure 6 d-f and g-i)). If age to maturity does not affect adult mortality, then the slopes of  $d(\alpha)$  and  $d_h(\alpha)$  will be zero, and the adult mortality rate imposes no selection on age to maturity [6].

To understand how changes in adult mortality can have these contrasting effects on age to maturity, it is helpful to consider the situation before the intervention is imposed. At the optimum age to maturity,  $\alpha^*$ , there is the highest possible product from the three components of fitness: (i) chance of surviving to maturity, (ii) fecundity and (iii) duration of reproduction (adult life expectancy). By definition, worms maturing earlier or later than the optimum age will not have maximum fitness, so any associated improvement in one or more of the fitness components must be proportionately more than offset by a reduction in the other component(s). For example, worms beginning reproduction after the optimum age will have a relative fitness benefit from increased fecundity, but this benefit must be outweighed by a proportionately greater reduction in the product of their chance of surviving to maturity and their duration of reproduction. Now consider an intervention which changes adult mortality rates and hence duration of reproduction, whilst the other two components of fitness remain unchanged. The proportionate rate of change in the duration of reproduction with increasing age to maturity may (i) remain unchanged, (ii) increase (adult life expectancy increasing more quickly, or decreasing more slowly with size than without the intervention), or (iii) reduce (adult life expectancy increasing more slowly or decreasing more rapidly with size than without the intervention). In case (i), the proportionate change in fitness costs and benefits for worms maturing before or after  $\alpha^*$  will be unchanged and the optimum age at maturity will be unaffected by the intervention. In case (ii), worms maturing after  $\alpha^*$ , will enjoy a greater proportionate improvement in

reproductive life than was the case with no intervention. Since the other components of fitness are unchanged, this means that increased fitness will now be achieved by worms maturing some time after  $\alpha^*$ , and such worms will be favoured by selection. In case (iii), the reverse occurs and selection will therefore favour earlier maturing worms.

**Figure 7 Illustration of increased adult mortality selecting for higher age at maturity**  
 In Panel A, graphs show survival, fecundity and life expectancy values for given mortality and fecundity functions. In panel B, graphs show survival, fecundity and life expectancy values for mortality and fecundity functions reflecting an intervention increasing adult mortality rates. Although adult life expectancy with the intervention is lower for all possible values of  $\alpha$ , the reduced rate of change in adult life expectancy around  $\alpha^*$  means that selection favours later maturity, exploiting the improved combination of survival and fecundity previously offset by reductions in life-expectancy values for higher values of  $\alpha$

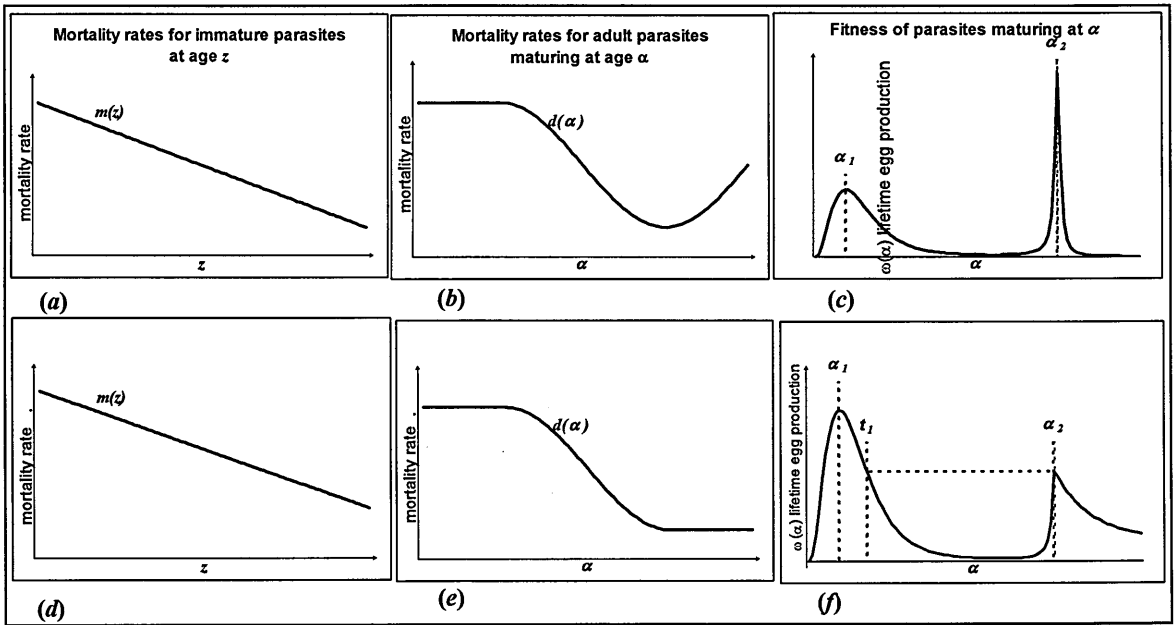


As an example, consider parasites evolved to mature at the optimum age in hosts whose immune response increases in effectiveness with the size of adult worms. An intervention increasing adult mortality for adult worms of all sizes, as might chemotherapy, which had a

more pronounced effect on smaller worms, or was size-independent, would decrease the proportionate reduction in life expectancy for later maturing worms, whilst leaving unchanged the proportionate increase in fecundity, and reduction in chance of reaching maturity. This sort of intervention would favour worms with longer times to maturity as illustrated in Figure 7.

There can also be situations in which there are more than one age to maturity associated with fitness maxima (Figure 8).

**Figure 8: Illustrations of multiple maxima for the fitness function (equation (7)).** Mortality rates as a function of age for juveniles (left panels) and of age at maturity for adults (middle panels) generate the fitness functions shown in the right hand panels. The adult mortality function shown could arise if, for example, bigger worms are harder to kill and smaller worms are harder to detect. For (c), multiple local optima are found, with the global optimum falling on the later peak at  $\alpha_2$ . In (e), there are also multiple local optima, but the global optimum falls at  $\alpha_1$ , on the first peak. In this case, in the absence of lower limits on the time needed to physically achieve maturity, selection would favour maturity at  $\alpha_1$ . If minimum achievable time to maturity is between  $\alpha_1$  and  $t_1$ , selection will favour maturity at the minimum achievable age, and if the minimum achievable time to maturity is greater than  $t_1$ , then selection will favour maturity at  $\alpha_2$ .

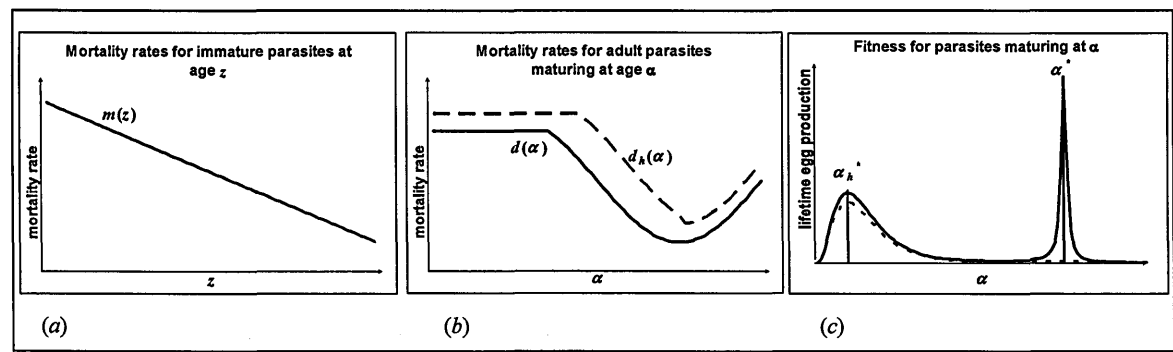


The situation is further complicated because the direction of initial selection pressure as given by the sign of equation (9) need not indicate the overall direction of selection in cases where multiple local optima exist for the fitness function under an intervention,  $\omega_h(\alpha)$ . In such cases, one of which is illustrated in Figure 9, the slope of  $\omega_h(\alpha)$  close to the original  $\alpha^*$  may not correspond to the change in  $\alpha$  required to give the maximum

achievable fitness. Outcomes in such cases will be unpredictable, depending upon specifics of starting conditions and the details of the functions involved.

**Figure 9: Illustration of the effects of an intervention changing adult mortality in an example with multiple optima for the fitness function.**

Panel (a) shows the assumed pre-maturity mortality function, panel (b) shows the assumed post maturity mortality functions with and without intervention, and panel (c) shows the fitness functions with and without the intervention. The slope of the post maturity mortality function under the intervention is always less than or equal to that without the intervention, so initial selection pressure will favour increased time to maturity. However, the overall optimum now falls on a different peak of the fitness function and selection will in fact favour a lower value of  $\alpha$ . Continuous lines show functions without the intervention, and dashed lines with the intervention.



## 2.6 Size-Dependent mortality function with pulsed interventions

In this section, we develop a model to study the effect of size-dependent mortality when there are pulsed interventions like regular drenching of farm animals with antihelminthics (henceforward “SDMP” model).

Drug treatments can arise as brief periodic events rather than on-going changes to mortality functions or fecundity parameters. Vaccine boosts (and some natural immunity processes) conceivably could do the same thing. The following assumptions and revised equations incorporate pulsed interventions, or interventions conferring transient changes in mortality, within the SDM model

- 7 Dosing is periodic at a fixed interval,  $I$ .
- 8 Parasites are assumed to infect hosts randomly at a constant rate, and are thus equally likely to arrive in host at any time point during the interval between dosing events.

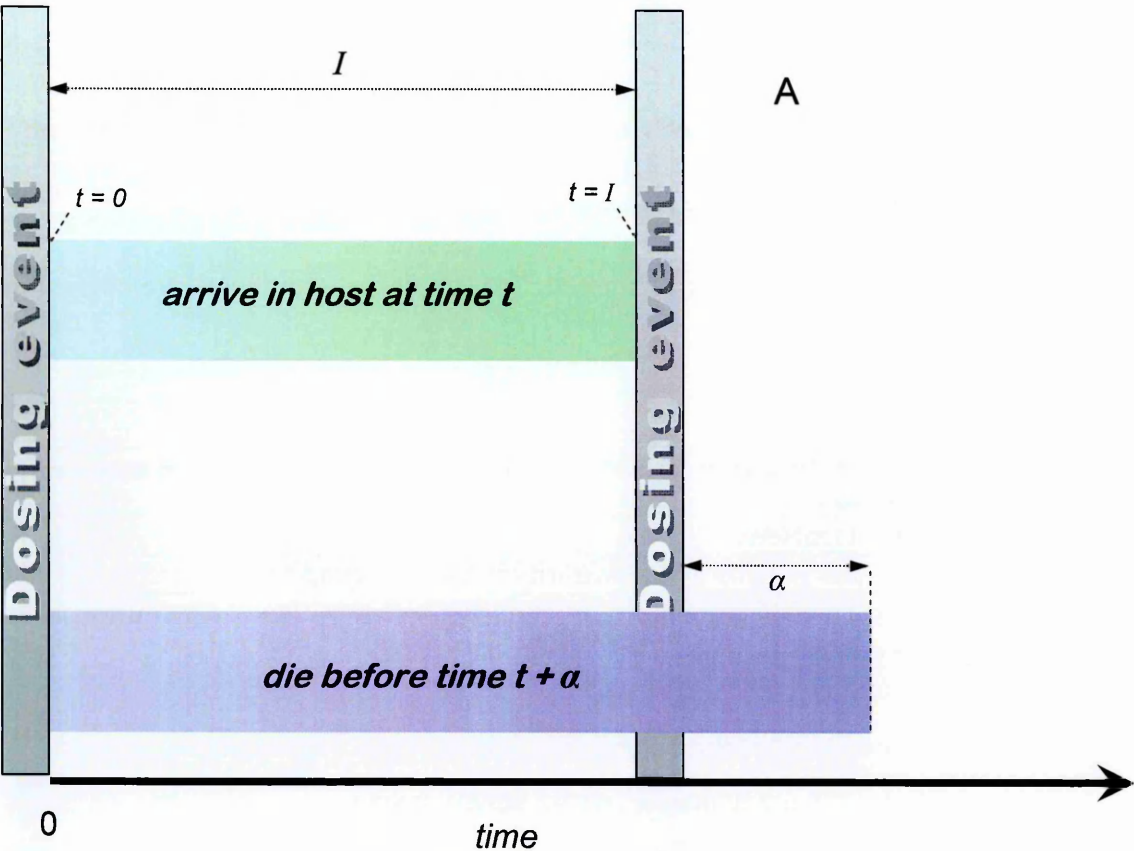
- 9 The proportion of parasites experiencing a second dose is assumed to be zero or very small for convenience of analysis (parameter values must be consistent with this assumption).
- 10 The effect of the intervention on any given parasite is assumed to vary only according to whether the parasite is immature or adult, irrespective of size or age.
- 11 Between dosing events, mortality rates are in accordance with those given by  $m(z)$  and  $d(\alpha)$ .

Table 3: Variables and Parameters for SDMP model. Note all ages are measured from first infection of the mammalian host.	
$\alpha$	age at maturity
$c$	constant relating age at maturity to worm fecundity
$\beta$	exponent of allometric relationship relating age at maturity to fecundity
$m(z)$	mortality rate experienced by juvenile parasites at age $z$
$d(\alpha)$	mortality rate experienced by adult parasites which matured at age $\alpha$
$s_p(\alpha^*)$	Selection gradient at $\alpha^*$ under pulsed dosing
$I$	time interval between doses; ( $I > \alpha$ )
$H$	proportion of hosts dosed during dosing events
$D_j$	probability of juvenile parasites dying if in dosed host
$D_m$	probability of adult worms dying if in dosed host
$t$	time from start of interval between dosing events ( $0 < t < I$ )
$\omega_p(\alpha)$	overall average fitness of parasites maturing at age $\alpha$ under pulsed dosing

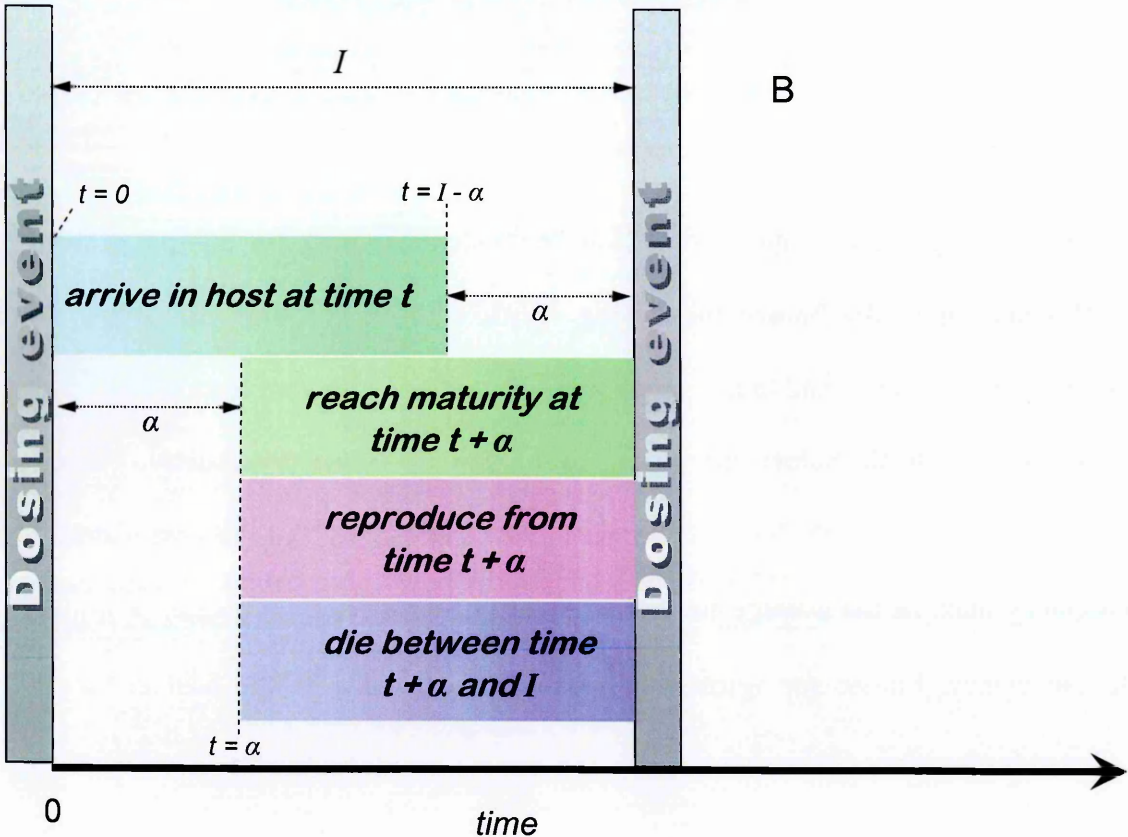
Worms infecting a host during interval  $I$  can be divided into the following four groups.

- A Worms which die before the dosing event, without reaching maturity.** These worms have zero fitness and thus do not contribute to the overall fitness function.
- B Worms which die before the dosing event, having reached maturity.** These have fitness in accordance with the assumptions of the SDM model, but the post-maturity life expectancy must be the average for worms dying before  $I$ , not the overall post-maturity life expectancy. Fitness for worms in this category, arriving in the host at time  $t$ , is modelled by function  $f(t)$ .

**Figure 10 Illustrative timeline for worms in category A**  
Worms arrive in-host at time  $t$ , between  $t=0$  and  $t=I$ , and then die between time  $t$  and  $t + \alpha$ , before reaching maturity



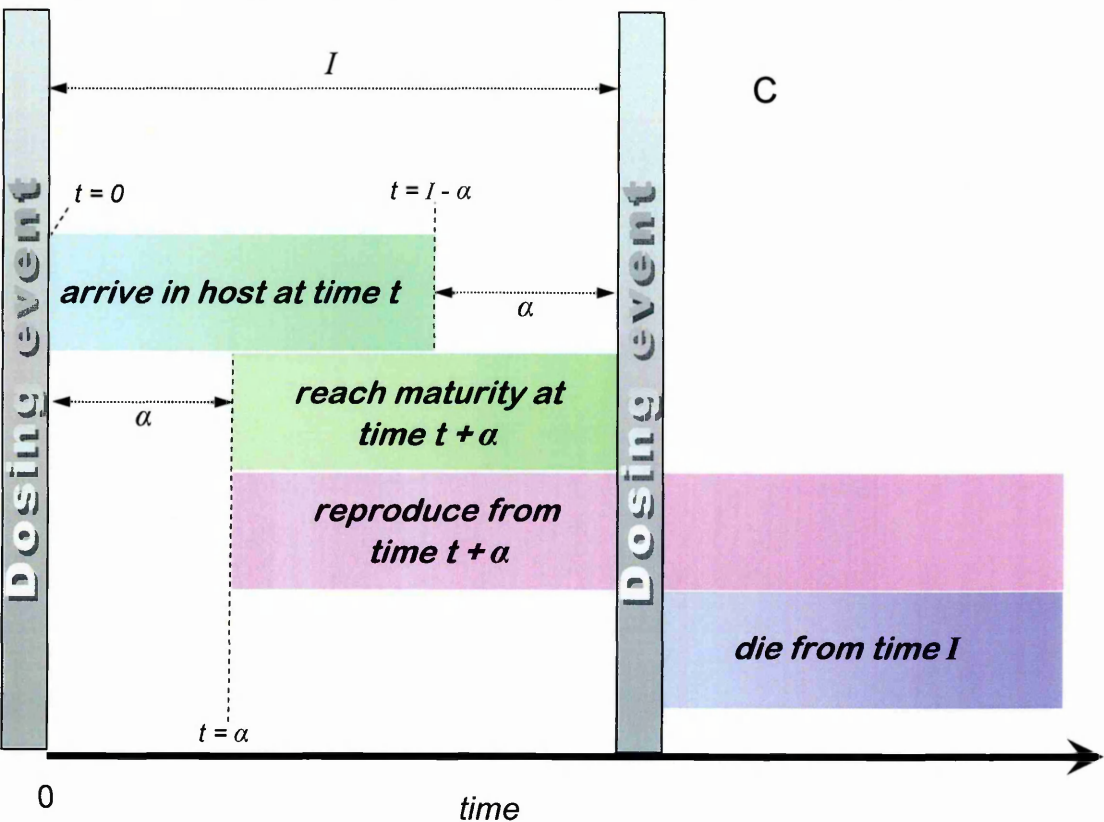
**Figure 11: Illustrative timeline for worms in category B**  
Worms arrive in-host at time  $t$ , between  $t = 0$  and  $t = I - \alpha$ , mature at time  $t + \alpha$ , and die before time  $I$ .



**C Worms which survive until the dosing event, and are mature at the time of the dosing event.** These worms will reproduce from maturity to age  $I - t$ , and then will either die in the dosing event, or will survive the dosing event and subsequently die according to the post-maturity mortality function. Fitness for worms in this category, arriving in the host at time  $t$ , is modelled by function  $g(t)$ .

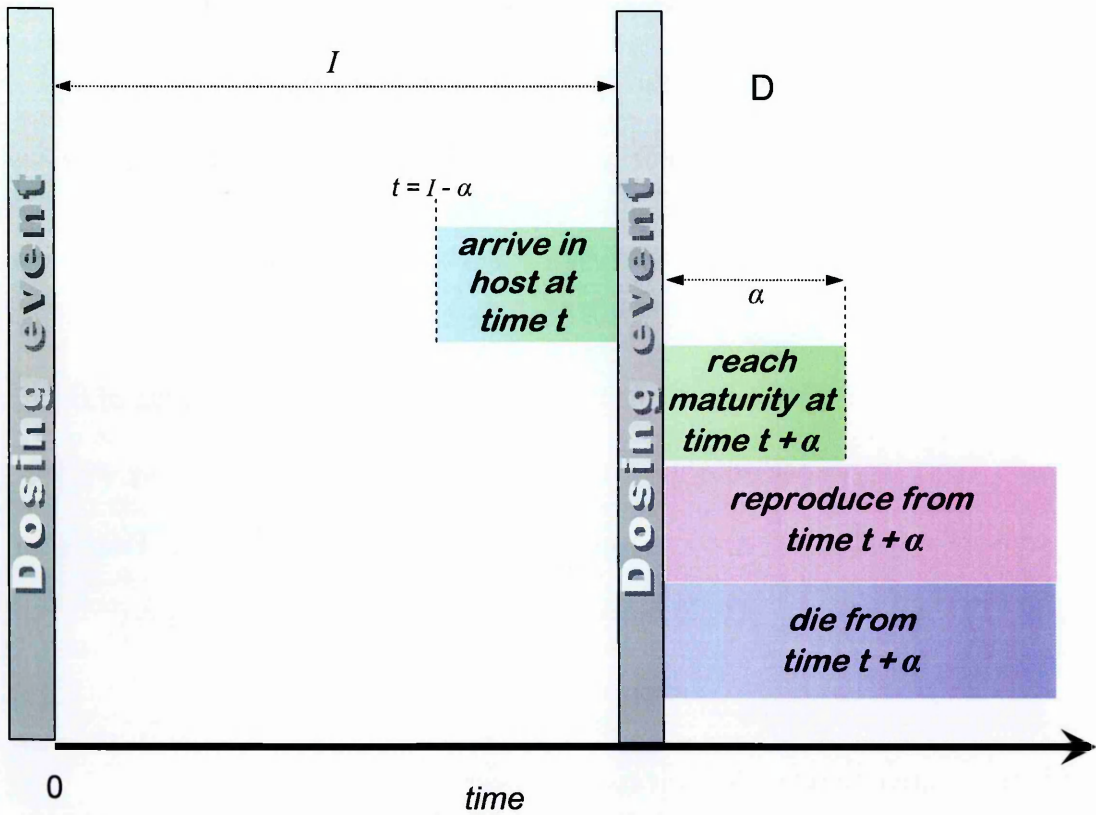
**D Worms which survive until the dosing event and are immature at the time of the dosing event.** These worms will either die in the dosing event before reproducing, or will survive to mature and reproduce in accordance with the SIM and SDM models. Fitness for worms in this category arriving in the host at time  $t$ , is modelled by function  $h(t)$ .

**Figure 12: Illustrative timeline for worms in category C**  
 Worms arrive in-host at time  $t$ , between  $t = 0$  and  $t = I - \alpha$ , mature at time  $t + \alpha$ , and survive to  $t = I$





**Figure 13 :Illustrative timeline for worms in category D**  
Worms arrive in-host at time  $t$ , between  $t = I - \alpha$  and  $t = I$ , and survive to mature at time  $t + \alpha$ .



Thus, for worms in category B, for  $0 < t \leq (I - \alpha)$  we have

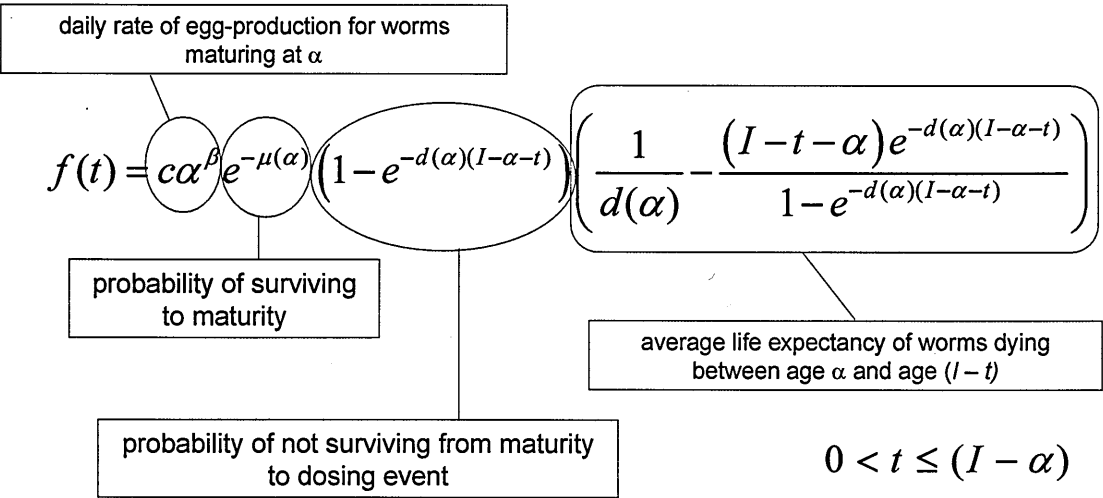
$$\begin{aligned}
 f(t) = & \text{probability of survival from } t \text{ to } t + \alpha \\
 & \times (1 - \text{probability of survival from } t + \alpha \text{ to } I) \\
 & \times \text{average life expectancy for worms dying between } t + \alpha \text{ and } I \\
 & \times \text{fecundity for worms maturing at age } \alpha
 \end{aligned}$$

The average life expectancy post maturity for worms born at time  $t$  which survive to time  $t + \alpha$  and die before time  $I$ , can be calculated from the definite integral on age  $q$ , measured from maturity, from 0 to  $(I - t - \alpha)$  of the proportion of such worms surviving to age  $q$  less the proportion which will survive to  $I$ .

Thus the average life expectancy post maturity, for worms born at time  $t$  which die between  $t + \alpha$  and  $I$  is

$$\frac{1}{1 - e^{-d(\alpha)(I-t-\alpha)}} \left( \int_0^{I-t-\alpha} e^{-d(\alpha)q} dq - (I-t-\alpha) e^{-d(\alpha)(I-t-\alpha)} \right) = \frac{1}{d(\alpha)} - \frac{(I-t-\alpha) e^{-d(\alpha)(I-t-\alpha)}}{1 - e^{-d(\alpha)(I-t-\alpha)}}$$

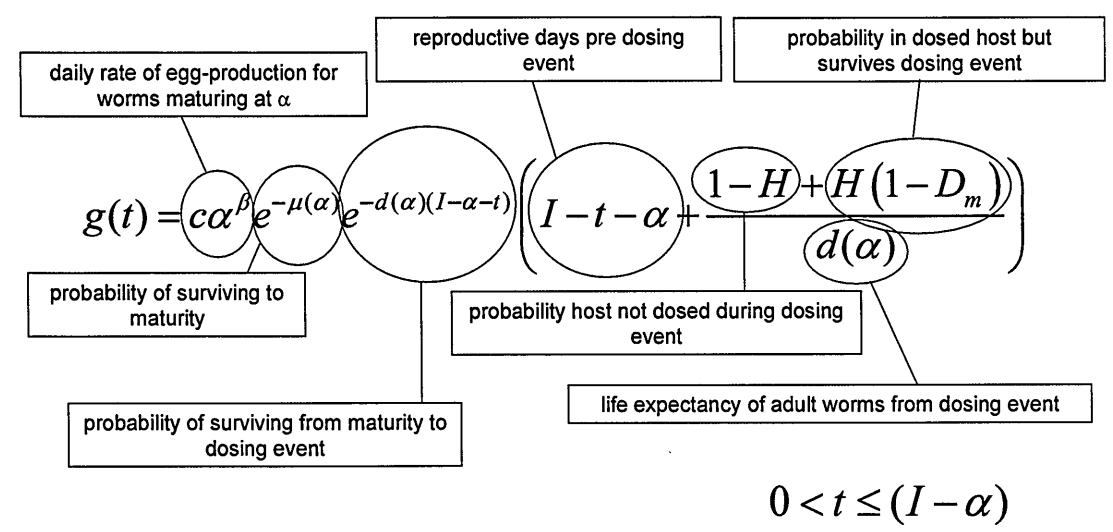
So



For worms in category C, we obtain, for  $0 < t \leq (I - \alpha)$

$$\begin{aligned} g(t) &= \text{probability of survival from } t \text{ to } I \\ &\times \left( (\text{probability in undosed host} + \text{probability in dosed host but survives}) \right. \\ &\times (\text{average life expectancy from } I) + I - \alpha - t \left. \right) \\ &\times \text{fecundity for worms maturing at age } \alpha \end{aligned}$$

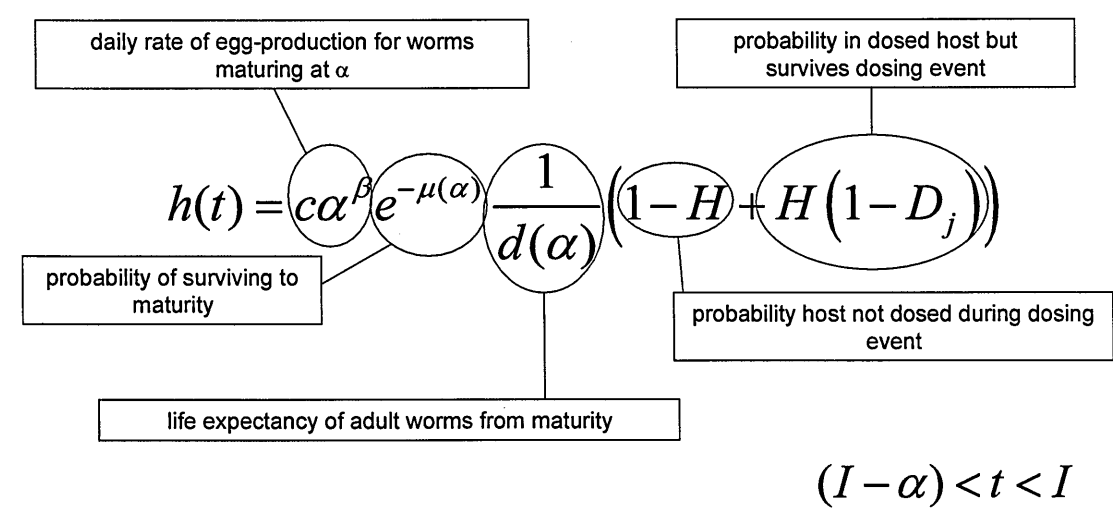
giving



For worms in category D we find, with  $(I - \alpha) < t < I$

$$\begin{aligned}
 h(t) &= \text{probability of survival from } t \text{ to } \alpha \\
 &\times (\text{probability in undosed host} + \text{probability in dosed host but survives}) \\
 &\times \text{average life expectancy from } \alpha \\
 &\times \text{fecundity for worms maturing at age } \alpha
 \end{aligned}$$

which yields



The definite integrals of these functions over the relevant ranges for  $t$  give the following;

$$\int_0^{I-\alpha} f(t)dt = \frac{c\alpha^\beta e^{-\mu(\alpha)}}{d(\alpha)^2} \left( (d(\alpha)(I-\alpha)+2)e^{-d(\alpha)(I-\alpha)} - 2 + d(\alpha)(I-\alpha) \right)$$

$$\int_0^{I-\alpha} g(t)dt = \frac{c\alpha^\beta e^{-\mu(\alpha)}}{d(\alpha)^2} \left( (2-HD_m) + (d(\alpha)(\alpha-I)+HD_m-2)e^{-d(\alpha)(I-\alpha)} \right)$$

$$\int_{I-\alpha}^I h(t)dt = \frac{c\alpha^{\beta+1} e^{-\mu(\alpha)} (1-HD_j)}{d(\alpha)}$$

These functions are then combined to give the average fitness for worms in all categories arriving at time  $t$

$$\begin{aligned} \omega_p(\alpha) = \frac{1}{I} & \left( \frac{c\alpha^\beta e^{-\mu(\alpha)}}{d(\alpha)^2} \left( (d(\alpha)(I-\alpha)+2)e^{-d(\alpha)(I-\alpha)} - 2 + d(\alpha)(I-\alpha) \right) \right. \\ & + \frac{c\alpha^\beta e^{-\mu(\alpha)}}{d(\alpha)^2} \left( (2-HD_m) + (d(\alpha)(\alpha-I)+HD_m-2)e^{-d(\alpha)(I-\alpha)} \right) \\ & \left. + \frac{c\alpha^{\beta+1} e^{-\mu(\alpha)} (1-HD_j)}{d(\alpha)} \right) \end{aligned}$$

This can be rearranged to give

$$\omega_p(\alpha) = \frac{c\alpha^\beta e^{-\mu(\alpha)}}{d(\alpha)} \left( 1 - \frac{H}{I} \left( D_m \frac{1 - e^{-d(\alpha)(I-\alpha)}}{d(\alpha)} + \alpha D_j \right) \right)$$

(10)

In order to find the optimum value of  $\alpha$  under the pulsed intervention,  $\alpha_p^*$ , we require

$\omega'_p(\alpha_p^*) = 0$ , which, since  $\frac{c\alpha_p^{*\beta} e^{-\mu(\alpha_p^*)}}{d(\alpha_p^*)}$  is non-zero, is equivalent to

$$\begin{aligned} 0 = & \left( \frac{\beta}{\alpha_p^*} - \frac{d'(\alpha_p^*)}{d(\alpha_p^*)} - m(\alpha_p^*) \right) \left( 1 + \frac{H}{I} \left( \frac{D_m \left( e^{-d(\alpha_p^*)(I-\alpha_p^*)} - 1 \right)}{d(\alpha_p^*)} - \alpha_p^* D_j \right) \right) \\ & + \frac{H}{I} \left( D_m \left( e^{-d(\alpha_p^*)(I-\alpha_p^*)} \left( 1 + \frac{d'(\alpha_p^*)}{d(\alpha_p^*)} \left( \alpha_p^* - I - \frac{1}{d(\alpha_p^*)} \right) \right) + \frac{d'(\alpha_p^*)}{d(\alpha_p^*)^2} \right) - D_j \right) \end{aligned}$$

From this equation it can be seen that, in addition to the detail of the underlying mortality functions  $m(z)$  and  $d(\alpha)$ , all the parameters associated with the pulsed intervention, the effectiveness of the treatment  $(D_m, D_j)$ , the proportion of the host population treated  $(H)$  and the interval between doses  $(I)$ , have the potential to affect the evolution of time to maturity.

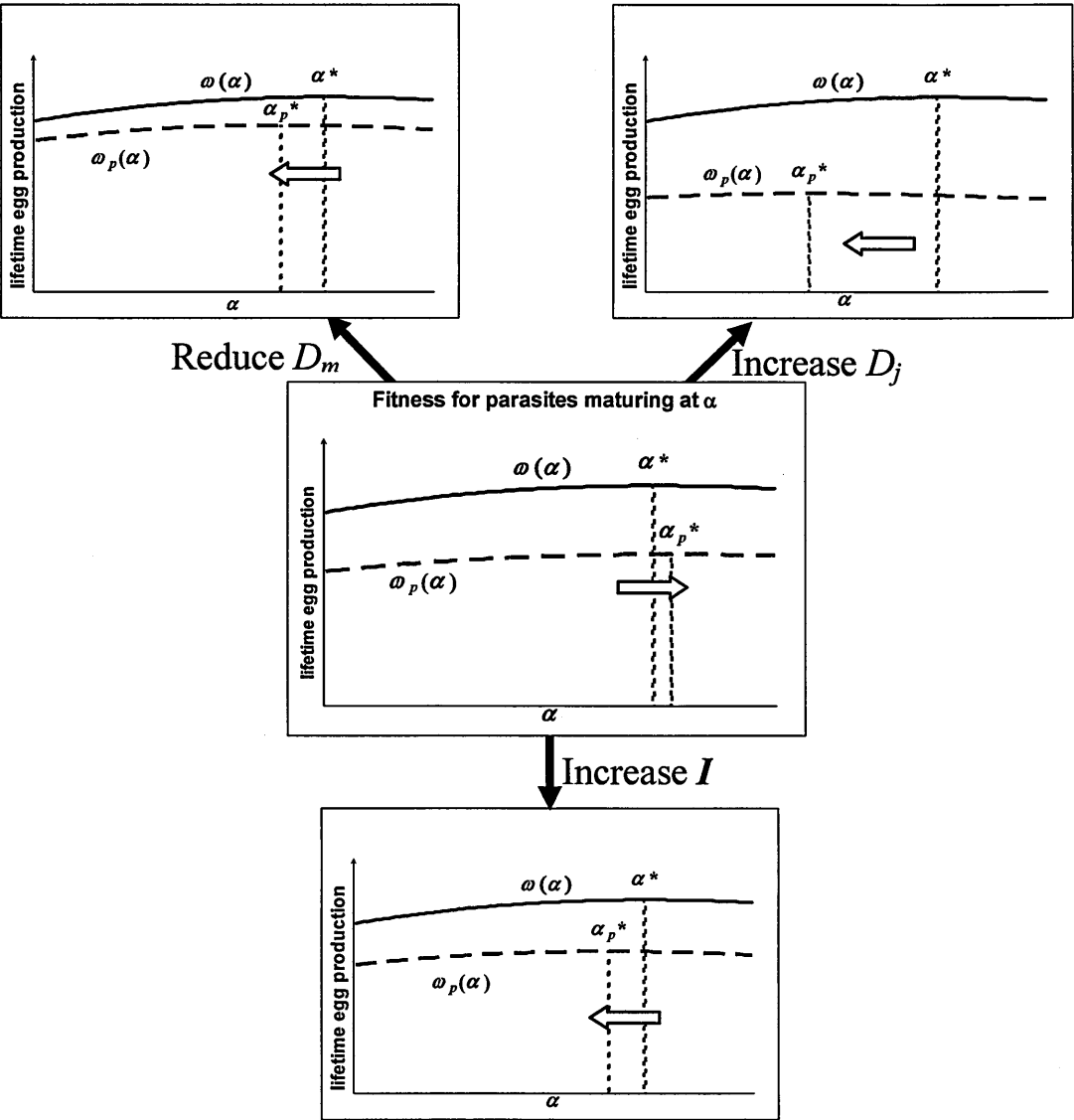
As for the SDM model, it is not possible to derive an explicit solution for  $\alpha_p^*$  for the SDMP model. However, again, the direction of the slope of the fitness function at  $\alpha^*$ , the optimum value of  $\alpha$  without the intervention, will give the direction of the initial selection pressure acting on time to maturity under the intervention. Since, from equation (8),  $\frac{\beta}{\alpha^*} - \frac{d'(\alpha^*)}{d(\alpha^*)} - m(\alpha^*) = 0$ , and since  $\frac{H}{I} \geq 0$ , the sign of the selection gradient at  $\alpha^*$  corresponds to the sign of  $s_p(\alpha^*)$ , where

$$s_p(\alpha^*) = D_m \left( e^{-d(\alpha^*)(I-\alpha^*)} \left( 1 + \frac{d'(\alpha^*)}{d(\alpha^*)} \left( \alpha^* - I - \frac{1}{d(\alpha^*)} \right) \right) + \frac{d'(\alpha^*)}{d(\alpha^*)^2} \right) - D_j \quad (12)$$

It is clear that the sign of  $s_p(\alpha^*)$  will depend upon the detail of the mortality functions and the parameters of the pulsed intervention and hence that selection pressure may favour increased or decreased  $\alpha$  according to the specifics of  $m(z)$  and  $d(\alpha)$ , and the values for the intervention parameters,  $D_j$ ,  $D_m$  and  $I$ . Given this, it is also clear that increasing the pre-maturity mortality  $D_j$  will always act to reduce the strength of selection for increased time to maturity when  $s_p(\alpha^*) > 0$ , and to increase the strength of selection for reduced time to maturity when  $s_p(\alpha^*) < 0$ .

**Figure 14: Illustration of the effects of values for dosing parameters on optimum time to maturity.**

From a given set of starting values, the direction of initial selection, towards longer or shorter time to maturity can be changed by adjusting any of the three parameters, dosing interval,  $I$ , treatment mortality in immature parasites,  $D_j$ , and in mature parasites,  $D_m$ . Simple linear functions are assumed for  $m(z)$  and  $d(\alpha)$ , with negative slope for  $d(\alpha)$ . Continuous lines show the fitness function without intervention,  $\omega(\alpha)$ , dashed lines show the fitness function under pulsed intervention,  $\omega_p(\alpha)$ .

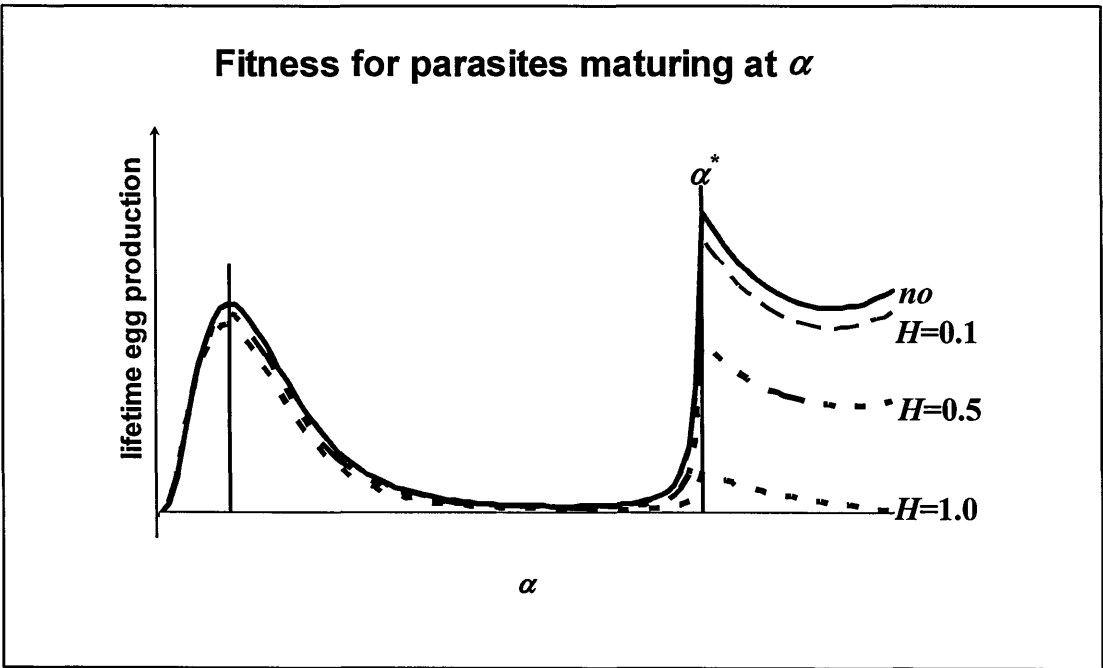


For example, Figure 14 illustrates that the optimum age to maturity under a pulsed intervention may be either longer or shorter than that without intervention, depending upon the relative and absolute values of the parameters  $D_j$ ,  $D_m$  and  $I$ . Thus, within a given range of values for any two of these parameters, the direction of initial selection can be determined by the value of the third parameter. For instance, within a suitable range of

values for  $I$  and  $D_m$ , changing the parameter  $D_j$  alone can change the direction of initial selection pressure. In each case, a limit may exist beyond which given values for one or more of these parameters fixes the direction of initial selection irrespective of the value of the others.

The proportion of hosts dosed,  $H$ , does not influence the direction of initial selection pressure. However, it does help to determine the size of the change from  $\alpha^*$  to  $\alpha_p^*$ , and can contribute to the overall direction of selection pressure in cases with multiple solutions as illustrated in Figure 15, where increasing  $H$  for a particular intervention produces very small changes in the values of  $\alpha$  at which the peaks of the fitness function fall, but ultimately causes the optimum value of  $\alpha$  to move from the second to the first peak. In practice, the outcome of such a change would depend *inter alia* upon there being sufficient variation in  $\alpha$  within the parasite population to allow the transition between the two optima, given that most intervening values of  $\alpha$  would be selected against.

**Figure 15: Effect of  $H$ , proportion hosts dosed, on selection for time to maturity.**  
 In this example with multiple optima for the fitness function, although the selection gradient around  $\alpha^*$  is positive and initial selection favours slightly increased time to maturity, sufficiently increasing the value of  $H$  moves the global optimum to the earlier peak, giving overall selection in favour of a reduced time to maturity.



## 2.7 Discussion

Nematode life history traits respond readily to selection [e.g. 39,44]. Consequently, animal and human health programmes which alter nematode mortality schedules (almost always the aim of such programmes) can drive life history evolution. For nematode age at maturity, a key life history trait with important fitness consequences, we find that the resulting evolution could have variable outcomes. In some cases selection for earlier maturity, giving smaller, less fecund worms, is potentially clinically beneficial. In some cases however, selection may favour later maturity giving larger worms producing more eggs with potentially clinically detrimental outcomes.

The models developed here show that when adult mortality rate changes with parasite size, both adult and juvenile mortality rates influence the evolution of age at maturity. Critically, and unlike juvenile mortality, the effect of adult mortality on optimal age to maturity is not unidirectional. Analysis of equations (8), and (9) shows that enhanced adult mortality can select for earlier or later age to maturity. Thus it is possible for animal or public health interventions like immunisation programmes or widespread chemotherapy to promote either smaller less fecund worms or larger more fecund worms.

Which of these possible outcomes occurs depends upon the biology of the parasite, the biology of the interactions between parasite and host immune system, and on the specifics of the health intervention applied. Predicting the outcome for any particular case requires knowledge of the pre- and post-maturity mortality functions, with and without the intervention. These are currently not known for any worm, and indeed, they would be difficult to determine even where direct experimentation is possible. Furthermore, for pulsed interventions, the interval between doses, the proportion of hosts dosed, and juvenile and adult parasite mortality rates resulting from the treatment all also help to determine whether selection will favour earlier or later maturing worms under the intervention. There are no simple generalities and indeed, given current levels of



understanding, it is not even easy to speculate on which evolutionary outcomes are more likely.

Nonetheless, since human interventions which change mortality schedules will exert selection pressure, we cannot simply ignore this issue. In many cases, the resulting evolution in life history traits will have little clinical significance, or will result in improved animal or public health outcomes. However, where, for example, the larval stage is much more pathogenic than the adult parasite, prolonging the time taken to reach adulthood may have undesirable clinical consequences. In such instances it would be important to take account of whether a given intervention strategy might be expected to select for a longer duration of larval stage, and plan accordingly.

In some instances, it may even be possible to avoid undesirable evolution. Often the selection pressures imposed by an intervention cannot be readily adjusted as, for example, with vaccine-induced immunity, although even here, the likely effects of stage or tissue-specific immunity could be investigated where there are several vaccine candidates being evaluated. However, for pulsed interventions, some elements, such as the time interval between doses, can readily be adjusted. Where such control is possible, rather than simply ameliorating selection for unwanted changes, it might be possible to specify an intervention to intentionally exert selection pressure in favour of a desirable change.

Detailed models developed to analyse specific cases could extend our models in a number of ways. For example, contrary to our assumption 11, worms which survive a dosing event may be damaged in some way and experience higher mortality rates, or have lower fecundity, than would otherwise be the case. This and other circumstances, such as seasonal life-cycles and dosing patterns might mean that worms are more likely to enter hosts early or late in the dosing cycle, contrary to our assumption 8. Certain combinations of dosing strategy and life-history may mean that a significant proportion of worms survive more than one dosing event, violating our assumption 9. Alternatively, density effects may mean that worms surviving a dosing event, or arriving in a host shortly after a dosing

event, may experience lower mortality or higher fecundity than would otherwise be the case. We doubt that such complexities would alter our general conclusion that some interventions can select for clinically-detrimental worm evolution, but they might nonetheless be important considerations for evaluating the magnitude and direction of such selection pressure in particular cases.

The relationship between mortality rate and age at maturity suggests that in an environment where mortality rate showed variation, as would be expected within a normal host population, there would be benefits to the parasite in adjusting the age of maturity according to the mortality rate actually experienced or predicted in its individual host, provided the benefits of such flexibility outweighed the costs of achieving it. Such flexibility has been demonstrated experimentally for at least three nematode species [45,46]. For example, *Litomosoides sigmodontis* size and reproductive strategy varies according to host immune response [46]. This may provide a means of testing our conclusions, by examining whether the changes flexibly adopted by worms under different mortality schedules, a system which should have evolved to maximise worm fitness, are consistent with the responses predicted by our models.

## 2.8 Conclusions

Interventions like chemotherapy, vaccination and, in the case of animal diseases, enhanced host resistance through selective breeding could affect many of the key functions and variables which shape the selection pressures on nematode age to maturity. Where mortality rates vary with worm size, we find that selection can be influenced by interventions changing either juvenile or adult mortality rates, and that, whilst changes to either can exert selection favouring earlier maturity, changes in adult mortality rates could also select for later maturing worms. This is in contrast to previous finding indicating that only interventions which increase juvenile mortality rates could drive selection on age at maturity, and only to produce earlier maturity.

For intermittent interventions, the timing of treatments, the proportion of hosts treated and the proportion of parasites killed in treated hosts can all influence selection on age at maturity. This opens the possibility of structuring dosing regimes to actively exert selection for clinically beneficial changes to age at maturity.

The clinical effects of extending or reducing the time taken for worms to mature, and hence increasing or reducing their adult size and fecundity, depend upon the specifics of parasite life-history within the host. Whether selection favours earlier or later age at maturity depends critically on the details of the parasite mortality functions with and without the intervention. Earlier optimism that health interventions would always prompt the evolution of smaller, less fecund and hence potentially clinically less damaging worms is premature. The detail matters, and our work suggests that when planning and implementing large-scale anti-helminth public health programs, the possible evolutionary consequences, beyond simple resistance, should be carefully considered.

*For ease of notation we must simplify,  
the mossie's a sphere that is able to fly,  
now assume a VC,  
set to infinity,  
and a cycle-length equal to pi.*

### 3 Chapter 3 Modelling Novel Public Health Interventions for Malaria Vectors

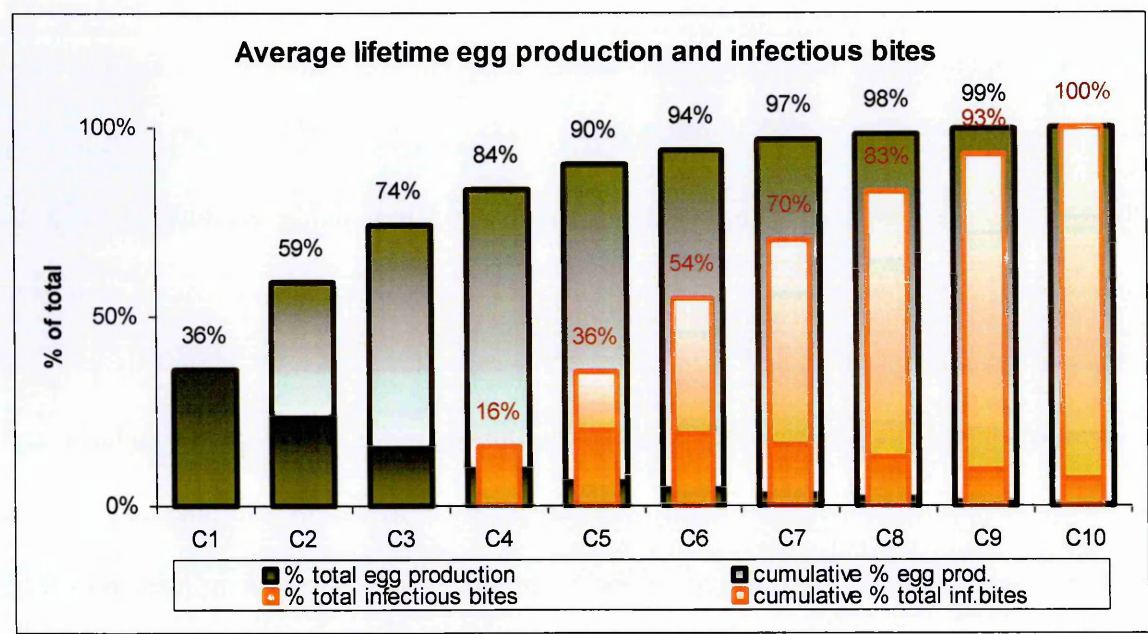
#### 3.1 Introduction

The reductions in global malaria burdens achieved by chemical insecticides against adult mosquitoes could be eroded by insecticide resistant mosquitoes [7,11-15,47], just as they were last century [48]. In principle, the evolution of insecticide resistance could be considerably slowed and perhaps prevented altogether by vector control aimed at killing only older mosquitoes (so-called 'late-life action', hereafter LLA) [49,50]). This exploits two features of the life histories of *Plasmodium*, the organism which causes malaria, and the mosquito vectors of the disease. *Plasmodium* parasites in a mosquito host take at least nine days to develop to a stage which can be transmitted to a human via an infectious bite, thus only older mosquitoes need to be removed to prevent disease transmission. Since mortality in wild mosquito populations is high, the majority of eggs are produced by young mosquitoes, so killing only older mosquitoes will have a relatively low impact on mosquito fitness, affecting the reproductive success only of the relatively few mosquitoes which survive to old age. Thus, a vector control treatment which kills only older mosquitoes could dramatically reduce transmission while exerting only weak selection for resistance (Figure 16).

We need to assess whether a balance is possible between useful transmission control and low selection for resistance and for this purpose we require a model which captures the detailed timings and probabilities of infection, infectiousness, reproduction and mortality over the mosquito lifespan. In order to encompass these elements, we have developed a model with two separate components, a markovian, deterministic, feeding

cycle model (FCM) which calculates survival, egg laying and infectious bite values for sequential age classes during the lifetime of an adult mosquito, and a population model (PM) which tracks the population-level spread of resistance alleles and corresponding loss of transmission control.

**Figure 16 Mosquito average lifetime egg production and infectious bites by age class**  
Average egg production and infectious bites from mosquitoes in age classes defined by gonotrophic cycle (C1, C2... C10) , expressed as percentages of total lifetime values. *Values calculated using FCM.*



We consider three types of health intervention, two kinds of LLA and a conventional instant-kill insecticide (CIKI). The LLAs either act as instant-kill insecticides which affect only mosquitoes above a given age (age-dependant insecticides, ADI), or time-delay insecticides (TDI), which kill according to a mortality schedule defined with respect to elapsed time from a mosquito’s first contact with the insecticide. TDIs may have simple mortality schedules comprising a delay of a fixed number of gonotrophic cycles until death, or more complex variable mortality schedules, such as might be generated by biopesticides (see chapters 4 and 5).

The model described below allows all interventions to be assessed assuming that mosquitoes contact them immediately following a blood meal from a human host. This would be consistent, for example, with indoor residual spraying (IRS), mosquitoes being

exposed to the intervention when resting on treated internal surfaces after feeding. CIKIs and ADIs can also be evaluated assuming a method of application which exposes feeding mosquitoes to the insecticide prior to biting, consistent with delivery via treated bed nets (ITNs). For TDIs, since they are always assumed to have some delay between contact and mortality, there is no difference, from the modelling perspective, between contact assumed before or after biting.

### 3.2 The Feeding Cycle Model

The FCM calculates survival, egg laying and infectious bite values across a series of discrete adult age classes for a specified type of mosquito (eg. susceptible) subjected to a given intervention (eg. a particular LLA insecticide at a particular coverage). Each sequential age class is defined as lasting for the average length of one gonotrophic cycle. Use of the mosquito feeding cycle as the basis for age-structured analyses of mosquito populations is a well-established methodology [51-54].

The FCM tracks possible states and transitions through each age class ( $i$ ), applying survival, exposure and infection probabilities (Figure 17). State changes depend on the preceding state, the passage of time, mortality rates and the probabilities of certain events, such as contacting an insecticide when resting after a human blood meal. Infection status for malaria, ( $m$ ), or a TDI, ( $l$ ), is zero for no infection, otherwise equal to the age of the infection.

The non-mathematical description of the model, incorporating both TDIs and ADIs, is as follows. Female mosquitoes are followed from successful emergence through ten gonotrophic cycles. In each cycle the probabilities of survival are tracked through the processes of host seeking, feeding, resting, finding an oviposition site and laying. For each cycle, the proportion of mosquitoes which acquire a malaria infection, contact a TDI, bite whilst infectious for malaria, and successfully lay eggs is also recorded. Specifically, the mosquito may die whilst searching for a host, with a probability arising from the time spent

searching, the background mortality rate, and any incremental mortality from a previously contacted TDI. If she survives searching, she then attempts to feed on a human with a given probability, and on a non-human with one minus that probability. She may die whilst attacking the host immediately before or immediately after feeding, with probabilities calculated from the underlying risk of death when attacking a host, and the probability of encountering an insecticide (CIKI or ADI) which kills on contact. Of those which successfully feed on a human host, females carrying a mature malaria infection give an infectious bite, whilst those so far uninfected may become infected, with a fixed probability. Those which survive feeding have a fixed probability of contacting a TDI, after which death may occur during resting with a probability calculated from the time spent resting, the background mortality rate, and any previous TDI exposure. Those surviving resting may die whilst searching for an egg-laying site, again depending on time and relevant mortality rates, and survivors may then die whilst attempting to lay, either before or after laying, with fixed probabilities. The tracked values give the proportion of mosquitoes surviving, biting and laying in each cycle and the proportion of mosquitoes starting each cycle with each possible combination of malaria and TDI status. This methodology is summarised in Figure 17. For example, for a case analysing the effects of a TDI, a mosquito commencing its fourth cycle with an infectious, three-cycle old malaria infection, and no previous exposure to an insecticide, will spend a defined period of time searching for a host, with an associated probability of dying from background mortality while it does so. It will then attack a host, with a given probability that the selected host will be a non-infectious human, a malaria-infectious human, or non-human, and a given probability of being killed whilst attacking the host before biting. If it survives to bite, and if the host is human, this is recorded as an infectious bite. There is then a given probability that it is killed by the host after biting. If it is not killed, it begins a period of resting, during which, if the chosen host was human, it has a fixed probability of being exposed to a TDI, as well as a given probability of dying from background mortality before leaving to

search for a laying site. During the search for a laying site there is a given probability that the mosquito may die from background mortality, or from the effects of its newly acquired contamination with the TDI. If it survives searching for a laying site it may die before or after laying with given probabilities. If still alive at the end of the cycle, it begins its fifth cycle with an infectious four-cycle old malaria infection, and a one-cycle old TDI exposure. For a case analysing the effects of an ADI or CIKI, the analysis would include a probability of contacting the pesticide before or after biting the host, and a probability of death, assumed to be instant, resulting from that contact, with zero probability of contacting a TDI.

The probability that a mosquito contacts and is affected (killed or infected) by a conventional or biological insecticide is input as a single 'coverage' value, incorporating the probabilities of being in a treated property, of contacting the pesticide, and of being affected by the pesticide during contact, with an appropriate adjustment for ADIs contacted by mosquitoes below the age at which the ADI becomes effective.



**Calculation of possible outcomes for feeding cycle  $i+1$  of the model, assuming status when commencing cycle  $i+1$  is age =  $i$ , malaria status =  $m_i$ , TDI status =  $l_i$ , with  $l_0=0$  and  $m_0=0$ . Each arrow represents a probability calculated by the model. If the malaria infection status (number of cycles since infection) of a mosquito when biting is greater than the development time of the malaria parasite in the mosquito, then ‘bites host’ after attacking a human host is recorded as an infectious bite.**



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behaviour, background mortality or fecundity in infected mosquitoes, and mosquitoes which survive and lay eggs whilst carrying a TDI are assumed to lay as many eggs at each laying event as uninfected individuals.

Late-life acting (LLA) insecticides might have many patterns of effectiveness. They could, for example, kill on contact, but affect only mosquitoes above a certain age. They might contaminate mosquitoes on contact and kill later, either after a fixed period of time, according to given mortality schedule, or when a contaminated mosquito reaches a certain age. Alternatively a contaminating LLA might have no effect until multiple doses have been acquired. Combinations of these modes might also be possible, for example, a contaminant LLA killing only mosquitoes above a certain age might require a development period after contamination before it causes mortality. For modelling purposes we consider a simplified sub-set of possible LLA modes of action: (i) killing on contact only mosquitoes above a certain age, e.g. a 4-cycle ADI kills only mosquitoes which contact it after having been through four or more cycles; (ii) killing a fixed number of cycles after contact, eg. a 4 cycle TDI kills mosquitoes four cycles after initial contact; (iii) killing according to a detailed mortality schedule from time of infection, as might be the case for a biopesticide. With a pre-bite delivery system, (i) and (ii) are equivalent if the TDI delay is equal to the ADI effective age (see Box 1).

<b>Box 1 Comparison of time-delay and age-dependant LLA insecticides</b> Time-dependent insecticides and age-dependent insecticides have equivalent effects on disease transmission and resistance evolution when ADI contact with the insecticide is assumed to occur pre-bite. Comparisons are made with mortality occurring 4 cycles after contact (TDI) or in mosquitoes aged 4 cycles or older (ADI).										
<b>Comparison of mortality arising from Age-Linked (ALI) and Time Delay (TDI) Insecticides</b> Both at 80% exposure levels, with mortality 4 cycles after contact (TDI) or in mosquitoes aged 4 cycles or older (ALI)										
	cycle number									
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
<b>Time Delay Treatment</b>	No mortality from treatment, no mosquitoes carrying TDI for 4 cycles			Of the mosquitoes surviving to cycle 4, 80% were exposed to TDI in cycle 1, and will now die as a result      Of the mosquitoes surviving to cycle 5, 80% were exposed to TDI in cycle 2, and will now die as a result      and so on.....						
Mortality in cycle from LLA										
	0.0%	0.0%	0.0%	80%	80%	80%	80%	80%	80%	80%
<b>Age-Linked Treatment</b>	No mortality from treatment, no mosquitoes 4 cycles or older			Of the mosquitoes surviving to cycle 4, 80% are now exposed to ADI, of which all are 4 cycles of age or older, and will die as a result      Of the mosquitoes surviving to cycle 5, 80% are now exposed to ADI, of which all are 4 cycles of age or older, and will die as a result      and so on.....						
Mortality in cycle from LLA										
	0.0%	0.0%	0.0%	80%	80%	80%	80%	80%	80%	80%

3.2.2 Feeding Cycle Model detail

Both CIKI and LLA pesticides offer public health benefits by reducing the numbers of mosquitoes that survive to give infectious bites in a treated population. Clearly the extent to which a reduction in infectious bites maps to reduced transmission and reduced numbers or severity of malaria cases in a human host population involves many complex, context-specific factors. For comparative purposes, however, it is assumed that in a given context, a given reduction in infectious bites will generate the same reduction in malaria transmission and hence malaria-induced morbidity and mortality, irrespective of the type of intervention from which it results. For generality, therefore, the comparative public health benefits of the insecticides considered in this analysis are all evaluated based on the reduction in infectious bites which they provide. This is quantified in the FCM for a given mosquito phenotype subject to a specific intervention by calculating RAIB, the proportionate reduction in the average number of infectious bites per mosquito per lifetime (AIB), defined as

$$RAIB = 1 - \frac{\text{AIB with treatment}}{\text{AIB without treatment}}$$

Assuming that the rate at which newly maturing adults join a population is constant through time, and that the size of the human host population is unaffected by the intervention being assessed, RAIB is equal to the proportionate reduction in the entomological inoculation rate (EIR), the number of infectious bites experienced per person per unit of time.

To evaluate mosquito fitness we use the average number of eggs produced per mosquito per lifetime as a proxy for lifetime reproductive success (LRS). The selection coefficient, the proportionate fitness benefit of resistance to a given intervention, is calculated as

$$\text{Selection Coefficient} = 1 - \frac{\text{LRS for specified mosquito type with intervention}}{\text{LRS for susceptible mosquitoes without intervention}}$$

A selection coefficient of zero means no selection pressure in favour of resistance, with higher selection coefficients indicating increasingly strong selection for resistance. Where no cost of resistance is assumed, the LRS for resistant mosquitoes is assumed to equal that for susceptible mosquitoes in the absence of any intervention.

Formulating these key results in relative terms minimises the sensitivity of our conclusions to parameter values which are independent of the vector control treatment or mosquito phenotype being evaluated.

With variables as defined in Table 4 the average number of eggs laid in a given cycle by mosquitoes surviving to the start of that cycle,  $F_i$ , is calculated as

$$F_i = \frac{\left( \sum_{m=0}^{i-1} \sum_{l=0}^{i-1} f_{i,m,l} v_{i,m,l} \right)}{V_i}$$

This provides the basis for the evaluation of relative fitness using a comparison of values

for  $\varphi$ , lifetime egg production, representing LRS,  $\varphi = \sum_{i=1}^{\lambda} F_i V_i$ .

Comparative levels of disease control are assessed using  $u$ , the average number of infectious bites per mosquito lifetime,  $u = \sum_{i=1}^{\lambda} I_i V_i$ .

The average number of infectious bites in a given cycle per mosquito alive at the start of the cycle,  $I_i$ , is calculated as

$$I_i = \frac{\sum_{m=D}^{i-1} \sum_{l=0}^{i-1} q_{i,m,l,2} v_{i,m,l} + q_{i,m,l,3} v_{i,m,l}}{V_i} \qquad i > D$$

(13)

The average probability of survival from start of cycle  $i$  to start of cycle  $(i + 1)$  is  $S_i$ , with

$$S_i = \frac{\left( \sum_{m=0}^{i-1} \sum_{l=0}^{i-1} s_{i,m,l} v_{i,m,l} \right)}{V_i}$$

(14)

**Table 4: Feeding Cycle Model parameters and variables**

Variable or Parameter	Symbol	Comments and Constraints
Time, measured in whole units equal to average length of sporogonic cycle, from infection of mosquito by malaria to cycle from which mosquito gives infectious bites	$D$	<i>input</i> $0 < D$
Number of age classes included in analysis	$\lambda$	
Cycle number ( <i>identifies specific cycle in the <math>\lambda</math> cycles over which probabilities are tracked in the FCM</i> )	$i$	$0 \leq i \leq \lambda$
Malaria status, the number of whole or partial cycles since infection with malaria	$m$	$0 \leq m \leq \lambda$ <i>m = 0 means not infected</i>
TDI status, the number of whole or partial cycles since being exposed to and acquiring a TDI	$l$	$0 \leq l \leq \lambda$ <i>l = 0 means not contaminated</i>
Average number of eggs laid in cycle $i$ by mosquitoes surviving to the start of cycle $i$	$F_i$	
Average lifetime number of eggs laid per mosquito	$\varphi$	

Variable or Parameter	Symbol	Comments and Constraints
Average number of eggs laid in cycle $i$ , by mosquitoes starting cycle $i$ with malaria status $m$ and TDI status $l$	$f_{i,m,l}$	$m < i \quad l < i$
Average probability of survival from start of cycle $i$ to start of cycle $i+1$	$S_i$	
Average probability that a mosquito starting cycle $i$ with malaria status $m$ and TDI status $l$ , will survive to start of cycle $i+1$	$s_{i,m,l}$	$m < i \quad l < i$
Average probability of a mosquito being alive at start of cycle $i$ .	$V_i$	
Average probability of a mosquito being alive, with malaria status $m$ and TDI status $l$ , at start of period $i$ .	$v_{i,m,l}$	$m < i \quad l < i$
Probability that a mosquito alive at start of cycle $i$ with malaria status $m$ and TDI status $l$ , survives and bites host type $h$ in cycle $i$	$q_{i,m,l,h}$	$m < i \quad l < i$
Type of host attacked	$h$	$h=1$ , non-human $h=2$ , non-infectious human $h=3$ , infectious human
Average number of infectious bites in cycle $i$ per mosquito alive at the start of cycle $i$	$I_i$	
Average lifetime number of infectious bites per mosquito	$u$	
Base instantaneous mortality rate per day for mosquito age $i$ , during activity B	$r_{B,i}$	input
Length of gonotrophic cycle (days)	$w$	input
Time spent host searching and feeding during a cycle (days)	$b$	input
Time spent finding oviposition site and laying during a cycle (days)	$\phi$	input
Length of resting period (days)	$\eta$	input
Proportion human population infectious for malaria	$p$	input
Probability attacks non-human host	$H$	input
Probability killed when attacking host before biting, with malaria infection aged $m$ (excluding mortality from insecticide treatments)	$a_{1,m}$	input
Probability killed when attacking host after biting, with malaria infection aged $m$ (excluding mortality from insecticide treatments)	$a_{2,m}$	input
Probability contacts and acquires a TDI whilst resting after biting human host (TDI 'coverage') (0 for cases assuming no TDI)	$X$	input
Probability acquires a <i>Plasmodium</i> infection when biting infectious human host	$M$	input
Probability contacts and is killed by instant-kill insecticide when attacking human host, before biting, in cycle $i$ . CIKI or ADI 'coverage' value. (0 for cases assuming no instant-kill intervention, or for age classes below the effective age of an ADI)	$k_{1,i}$	input
Probability contacts and is killed by instant-kill insecticide when attacking human host, after biting, in cycle $i$ . CIKI or ADI 'coverage' value. (0 for cases assuming no instant-kill intervention, or for age classes below the effective age of an ADI)	$k_{2,i}$	input
Number of eggs laid per successfully laying mosquito per cycle	$L$	input
Malaria-fecundity adjustment factor, proportionate number of eggs produced by mosquitoes with malaria infection age $m$	$E_{1,m}$	input
TDI-fecundity adjustment factor, proportionate number of eggs produced by mosquitoes with TDI status $l$	$E_{2,l}$	input
Probability that a mosquito alive at start of cycle $i$ with malaria status $m$ and TDI status $l$ , having survived to bite, then survives to lay eggs	$z_{i,m,l}$	$m < i \quad 0 < l < i$
Probability that a mosquito alive at start of cycle $i$ with malaria status $m$ and no existing TDI, having survived to bite, will survive to lay eggs if it acquires a new TDI in cycle $i$	$z^A_{i,m}$	
Probability that a mosquito alive at start of cycle $i$ with malaria status $m$ and no existing TDI, will having survived to bite, will survive to lay eggs if it does not acquire a new TDI in cycle $i$	$z^B_{i,m}$	
Instantaneous daily mortality rate from TDI on $x$ th day after infection, for mosquitoes with no malaria infection	$\beta_x$	input

Variable or Parameter	Symbol	Comments and Constraints
Instantaneous per day mortality rate from TDI on $x$ th day after infection, for mosquitoes with malaria infection	$\varepsilon_x$	input
Incremental daily mortality rate with malaria infection age $m$	$\gamma_m$	input
Incremental daily mortality rate assumed as cost of resistance	$\alpha$	input
% reduction in egg production assumed as cost of resistance	$\theta$	input
Differential mortality factor applied to TDI or ADI mortality for mosquitoes without a malaria infection	$\delta$	$0 \leq \delta \leq 1$
Activity type, searching for host, resting, searching for laying site	$B$	host-seeking = 1 resting = 2 site-seeking = 3
Probability of dying from action of TDI before biting host in cycle $i$ , for mosquito starting cycle $i$ with malaria status $m$ and biopesticide status $l$	$\sigma_{i,m,l}$	
Probability of dying from action of TDI between biting host and laying, in cycle $i$ , for mosquito starting cycle $i$ with malaria status $m$ and biopesticide status $l$	$\tau_{i,m,l}$	$l > 0$
Probability of dying from action of TDI between biting host and laying, in cycle $i$ , for mosquito starting cycle $i$ with malaria status $m$ and biopesticide status 0 and acquiring a new biopesticide infection during the cycle	$\tau_{i,m,0}$	

For the purposes of this evaluation, mortality specifically associated with egg-laying, if assumed equally divided pre and post egg-laying, would have an impact on the calculations identical to that for pre-and post feeding mortality. It is therefore not addressed separately in the model.

The average number of eggs laid in cycle  $i$ , by mosquitoes starting cycle  $i$  with malaria status  $m$  and TDI status  $l$  is defined as

$$f_{i,m,l} = L(1-\theta)E_{1,m}E_{2,l} \left( \sum_{h=1}^3 q_{i,m,l,h} \right) z_{i,m,l}$$

$l > 0$

$$f_{i,m,0} = L(1-\theta)E_{1,m}E_{2,0} \left( q_{i,m,0,1} z_{i,m}^B + \sum_{h=1}^2 q_{i,m,l,h} \left( (1-X) z_{i,m}^B + X z_{i,m}^A \right) \right)$$

(15)

The average probability of an adult mosquito surviving to the start of cycle  $i$ ,  $V_i$ , is 1 for cycle 1. For all subsequent cycles,  $V_i$  is the sum for all possible combinations of fungus & malaria status of the probabilities of an adult mosquito surviving to the start of cycle  $i$ .

$$V_1 = 1$$

$$V_i = \sum_{m=0}^{i-1} \sum_{l=0}^{i-1} v_{i,m,l} \quad i > 1$$

The various survival probabilities,  $v_{i,m,l}$  are calculated as follows. The average probability of an adult mosquito surviving to the start of cycle  $i$ , and being in the  $m$ th cycle of malaria infection and the  $l$ th cycle since contamination with a TDI at the start of cycle  $i$ ,  $v_{i,m,l}$ , is 1.00 at the start of cycle 1, and thereafter calculated for each possible combination of  $m$  and  $l$  at the start of the preceding cycle.

$$v_{1,0,0} = 1$$

The probability of surviving to the start of cycle  $i$  with no malaria or TDI,  $v_{i,0,0}$ , is the probability of surviving, uninfected, to the start of the previous cycle, and then surviving biting a non human host, or biting a human host without being infected by malaria or acquiring a TDI, and then surviving through laying.

$$v_{i,0,0} = v_{i-1,0,0} \left( q_{i-1,0,0,1} + \left( q_{i-1,0,0,2} + q_{i-1,0,0,3} (1-M) \right) (1-k_{2,i-1} \delta) (1-X) \right) z_{i-1,0}^B$$

$$i > 1$$

The probability of surviving to the start of cycle  $i$  with newly acquired TDI and malaria,  $v_{i,1,1}$ , is the probability of surviving, uninfected, to the start of the previous cycle, and then surviving biting an infectious human host, becoming infected with malaria and acquiring a TDI, and then surviving through laying, without being killed by any rapid TDI mortality.

$$v_{i,1,1} = v_{i-1,0,0} q_{i-1,0,0,3} \left( 1 - k_{2,i-1} \delta \right) M X z_{i-1,0}^A \quad i > 1$$

The probability of surviving to the start of cycle  $i$  with a newly-acquired malaria infection,



and no new TDI contamination,  $v_{i,1,0}$ , is the probability of surviving, uninfected, to the start of the previous cycle, and then surviving biting an infectious human host and becoming infected by malaria, not acquiring a TDI, and then surviving through laying.

$$v_{i,1,0} = v_{i-1,0,0} q_{i-1,0,0,3} M (1 - k_{2,i-1} \delta) (1 - X) z_{i-1,0}^B \quad i > 1$$

The probability of surviving to the start of cycle  $i$  with a newly-acquired malaria infection, and an existing TDI,  $v_{i,1,l}$ , is the probability of surviving, with a TDI, but no malaria infection, to the start of the previous cycle, and then surviving biting an infectious human host and becoming infected by malaria and then surviving through laying, with survival probabilities reflecting additional mortality from the TDI.

$$v_{i,1,l} = v_{i-1,0,l-1} q_{i-1,0,l-1,3} (1 - k_{2,i-1} \delta) M z_{i-1,0,l-1} \quad i > 1 \quad l > 1$$

(16)

The probability of surviving to the start of cycle  $i$  with an existing malaria infection, and no TDI,  $v_{i,m,0}$ , is the probability of surviving, with a malaria infection but no TDI, to the start of the previous cycle, and then surviving biting a non-human host or biting a human host without acquiring a TDI, and then surviving through laying.

$$v_{i,m,0} = v_{i-1,m-1,0} (q_{i-1,m-1,0,1} + (q_{i-1,m-1,0,2} + q_{i-1,m-1,0,3}) (1 - k_{2,i-1}) (1 - X)) z_{i-1,m-1}^B$$

$i > 1 \quad m > 1$

The probability of surviving to the start of cycle  $i$  with no malaria infection, and a newly acquired TDI,  $v_{i,0,1}$ , is the probability of surviving, with no malaria or TDI, to the start of the previous cycle, and then surviving biting a human host, not acquiring a malaria infection and acquiring a TDI, and then surviving through laying, without being killed by any rapid TDI mortality.

$$v_{i,0,1} = v_{i-1,0,0} (q_{i-1,0,0,2} + q_{i-1,0,0,3} (1 - M)) (1 - k_{2,i-1} \delta) X z_{i-1,0}^A$$

$i > 1$

The probability of surviving to the start of cycle  $i$  with an existing malaria infection, and a newly acquired TDI,  $v_{i,m,1}$ , is the probability of surviving, with a malaria infection but no TDI, to the start of the previous cycle, surviving biting a human host and acquiring a TDI, and then surviving through laying, without being killed by any rapid TDI mortality.

$$v_{i,m,1} = v_{i-1,m-1,0} (q_{i-1,m-1,0,2} + q_{i-1,m-1,0,3}) (1 - k_{2,i-1}) X z_{i-1,m-1}^A \quad i > 1 \quad m > 1$$

The probability of surviving to the start of cycle  $i$  with no malaria infection, and an existing TDI,  $v_{i,0,l}$ , is the probability of surviving, with no malaria infection and an existing TDI, to the start of the previous cycle, and then surviving biting a human host without acquiring a malaria infection, then surviving through laying, with survival probabilities reflecting additional mortality from the TDI.

$$v_{i,0,l} = v_{i-1,0,l-1} \left( q_{i-1,0,l-1,1} + (q_{i-1,0,l-1,2} + q_{i-1,0,l-1,3} (1 - M)) (1 - k_{2,i-1} \delta) \right) z_{i-1,0,l-1}$$

$i > 1 \quad l > 1$

(17)

The probability of surviving to the start of cycle  $i$  with existing malaria and TDI,  $v_{i,m,l}$ , is the probability of surviving, with existing malaria infection and TDI, to the start of the previous cycle, surviving biting any host, then surviving through laying, with survival probabilities reflecting additional mortality from the TDI.

$$v_{i,m,l} = v_{i-1,m-1,l-1} \left( q_{i-1,m-1,l-1,1} + (q_{i-1,m-1,l-1,2} + q_{i-1,m-1,l-1,3}) (1 - k_{2,i-1}) \right) z_{i-1,m-1,l-1} \quad i > 1 \quad m > 1 \quad l > 1$$

(18)

The probabilities of surviving through cycle  $i$  are calculated as follows. The average probability,  $s_{i,m,l}$ , that mosquitoes starting cycle  $i$  with any malaria status and an existing TDI, will survive to the start of cycle  $i + 1$  is calculated as the probability of surviving biting a non-human host, plus the probability of biting a human host without being killed by an instant-kill insecticide, and then surviving to lay, with survival probabilities reflecting additional mortality from the TDI

$$\begin{aligned}
s_{i,0,l} &= \left( (1 - \delta k_{2,i}) \sum_{h=2}^3 q_{i,m,l,h} + q_{i,m,l,1} \right) z_{i,m,l} \quad i < \lambda \quad l > 0 \\
s_{i,m,l} &= \left( (1 - k_{2,i}) \sum_{h=2}^3 q_{i,m,l,h} + q_{i,m,l,1} \right) z_{i,m,l} \quad i < \lambda \quad l > 0 \quad m > 0
\end{aligned}
\tag{19}$$

The average probability,  $s_{i,m,0}$ , that mosquitoes starting cycle  $i$  with any malaria status and no TDI, will survive to the start of cycle  $i + 1$  is calculated as the probability of surviving biting a non-human host, plus the probability of biting a human host without being killed by an instant-kill insecticide, and then either not acquiring a TDI, or acquiring a TDI but not being killed by it before the end of the cycle, and surviving to lay.

$$\begin{aligned}
s_{i,0,0} &= \left( (1 - \delta k_{2,i}) \sum_{h=2}^3 q_{i,m,l,h} \left( (1 - X) z_{i,m}^B + X z_{i,m}^A \right) + q_{i,m,l,1} z_{i,m}^B \right) \quad i < \lambda \quad l = 0 \\
s_{i,m,0} &= \left( (1 - k_{2,i}) \sum_{h=2}^3 q_{i,m,l,h} \left( (1 - X) z_{i,m}^B + X z_{i,m}^A \right) + q_{i,m,l,1} z_{i,m}^B \right) \quad i < \lambda \quad l = 0 \quad m > 0
\end{aligned}$$

The probabilities of surviving host seeking and biting in cycle  $i$ ,  $q_{i,m,l,h}$ , are calculated as follows. The probability,  $q_{i,m,l,1}$ , that a mosquito starting cycle  $i$  with malaria status  $m$ , and TDI status  $l$ , survives host seeking and biting a non-human host, is the proportion of non-human hosts multiplied by the probability of surviving background mortality and the effects of any previously acquired TDI whilst host-seeking, and successfully biting without being killed whilst attacking host,

$$q_{i,m,l,1} = H(1 - \sigma_{i,m,l}) e^{-b(r_{1,i} + \gamma_m + \alpha)} (1 - a_{1,m}).$$

The probability,  $q_{i,0,l,2}$ , that a mosquito starting cycle  $i$  with no malaria infection, and TDI status  $l$ , survives seeking and biting a human host not infectious for malaria is the proportion of hosts which are human and not infectious for malaria, multiplied by the probability of surviving background mortality and the effects of any previously acquired

TDI whilst host-seeking, not being affected by an instant-kill insecticide before biting, and successfully biting without being killed whilst attacking host is

$$q_{i,0,l,2} = (1-p)(1-H)(1-\sigma_{i,0,l})e^{-b(r_{1,i}+\gamma_m+\alpha)}(1-k_{1,i}\delta)(1-a_{1,m}).$$

The probability,  $q_{i,m,l,2}$ , that a mosquito starting cycle  $i$  with an existing malaria infection, and TDI status  $l$ , survives seeking and biting a human host not infectious for malaria is the proportion of hosts which are human and not infectious for malaria, multiplied by the probability of surviving background mortality and the effects of any previously acquired TDI whilst host-seeking, not being affected by an instant-kill insecticide before biting, and successfully biting without being killed whilst attacking host is

$$q_{i,m,l,2} = (1-p)(1-H)(1-\sigma_{i,m,l})e^{-b(r_{1,i}+\gamma_m+\alpha)}(1-k_{1,i})(1-a_{1,m}) \quad m > 0$$

The probability,  $q_{i,0,l,3}$ , that a mosquito starting cycle  $i$  with no malaria infection, and TDI status  $l$ , survives seeking and biting a human host infectious for malaria is the proportion of hosts which are human and infectious for malaria, multiplied by the probability of surviving background mortality and the effects of any TDI whilst host-seeking, and successfully biting without being killed whilst attacking host is

$$q_{i,0,l,3} = p(1-H)(1-\sigma_{i,0,l})e^{-b(r_{1,i}+\gamma_0+\alpha)}(1-k_{1,i}\delta)(1-a_{1,m}).$$

The probability,  $q_{i,m,l,3}$ , that a mosquito starting cycle  $i$  with malaria status  $m$ , and TDI status  $l$ , survives seeking and biting a human host infectious for malaria is the proportion of hosts which are human and infectious for malaria, multiplied by the probability of surviving background mortality and the effects of any TDI whilst host-seeking, and successfully biting without being killed whilst attacking host is

$$q_{i,m,l,3} = p(1-H)(1-\sigma_{i,m,l})e^{-b(r_{1,i}+\gamma_m+\alpha)}(1-k_{1,i})(1-a_{1,m}) \quad m > 0$$

The probabilities,  $Z_{i,m,l}$  that a mosquito starting cycle  $i$  with status  $m,l$ , will survive resting, site seeking and lay eggs are calculated as follows.

$$Z_{i,m,l} = (1 - a_{2,m}) (1 - \tau_{i,m,l}) e^{-(r_{3,i}\phi + r_{2,i}\eta + (\gamma_m + \alpha)(\phi + \eta))} \quad l > 0$$

Probability,  $Z_{i,m}^A$  that a mosquito starting cycle  $i$  with no previously acquired TDI, having survived biting a host, will survive resting, site seeking and lay eggs if it acquires a new TDI during cycle  $i$  is

$$Z_{i,m}^A = (1 - a_{2,m}) (1 - \tau_{i,m,0}) e^{-(r_{3,i}\phi + r_{2,i}\eta + (\gamma_m + \alpha)(\phi + \eta))}$$

Probability,  $Z_{i,m}^B$  that a mosquito starting cycle  $i$  with no previously acquired TDI will survive resting, site seeking and lay eggs if it does not acquire a new TDI infection is

$$Z_{i,m}^B = (1 - a_{2,m}) e^{-(r_{3,i}\phi + r_{2,i}\eta + (\gamma_m + \alpha)(\phi + \eta))}$$

The probabilities,  $\sigma_{i,m,l}$ , of dying from the effects of a TDI whilst host-seeking in cycle  $i$ , for a mosquito starting cycle  $i$  with infection status  $l$  are calculated (with  $\lfloor x \rfloor$  giving largest integer less than  $x$ ) as;

$$\sigma_{i,m,0} = 0$$

$$\sigma_{i,0,l} = 1 - e^{-\left( (\lfloor wl - b \rfloor + 1 - (wl - b)) \beta_{\lfloor wl - b + 1 \rfloor} + \left( \sum_{x=\lfloor wl - b + 1 \rfloor + 1}^{\lfloor wl \rfloor} \beta_x \right) + (wl - \lfloor wl \rfloor) \beta_{\lfloor wl + 1 \rfloor} \right)} \quad l > 0$$

$$\sigma_{i,m,l} = 1 - e^{-\left( (\lfloor wl - b \rfloor + 1 - (wl - b)) \varepsilon_{\lfloor wl - b + 1 \rfloor} + \left( \sum_{x=\lfloor wl - b + 1 \rfloor + 1}^{\lfloor wl \rfloor} \varepsilon_x \right) + (wl - \lfloor wl \rfloor) \varepsilon_{\lfloor wl + 1 \rfloor} \right)} \quad l > 0 \quad m > 0$$

The probability,  $\tau_{i,m,l}$ , of dying from the action of a TDI between biting and laying during cycle  $i$ , having started the cycle with infection status  $m,l$  is calculated for mosquitoes which have a previously acquired TDI at the start of cycle  $i$ , or which acquire a new TDI during cycle  $i$ , as follows;

$$\tau_{i,0,l} = 1 - e^{-\left( \left( \lfloor wl+1 \rfloor - wl \right) \beta_{\lfloor wl+1 \rfloor} + \sum_{x=\lfloor wl+1 \rfloor+1}^{\lfloor wl+\phi+\eta \rfloor} \beta_x + \left( wl+\phi+\eta - \lfloor wl+\phi+\eta \rfloor \right) \beta_{\lfloor wl+\phi+\eta+1 \rfloor} \right)}$$

$l > 0$

$$\tau_{i,m,l} = 1 - e^{-\left( \left( \lfloor wl+1 \rfloor - wl \right) \varepsilon_{\lfloor wl+1 \rfloor} + \sum_{x=\lfloor wl+1 \rfloor+1}^{\lfloor wl+\phi+\eta \rfloor} \varepsilon_x + \left( wl+\phi+\eta - \lfloor wl+\phi+\eta \rfloor \right) \varepsilon_{\lfloor wl+\phi+\eta+1 \rfloor} \right)}$$

$l > 0 \quad m > 0$

For mosquitoes newly infected during cycle  $i$ , the probabilities,  $\tau_{i,m,0}$ , of dying from the effects of a TDI before the end of the cycle are,

$$\tau_{i,0,0} = 1 - e^{-\left( \left( \sum_{x=1}^{\lfloor \phi+\eta \rfloor} \beta_x \right) + \left( \phi+\eta - \lfloor \phi+\eta \rfloor \right) \beta_{\lfloor \phi+\eta+1 \rfloor} \right)}$$

$$\tau_{i,m,0} = 1 - e^{-\left( \left( \sum_{x=1}^{\lfloor \phi+\eta \rfloor} \varepsilon_x \right) + \left( \phi+\eta - \lfloor \phi+\eta \rfloor \right) \varepsilon_{\lfloor \phi+\eta+1 \rfloor} \right)}$$

$m > 0$

For cases assessing differential TDI mortality for malaria-infected and uninfected mosquitoes,  $\beta_x = \varepsilon_x \delta$ .

### 3.3 Modelling the spread of resistance – the population model

The PM tracks susceptible and resistant phenotypes over a sequence of time periods for a

population subject to a given vector control treatment. The key outputs, calculated for each time period, are the proportion of the population with resistant and susceptible phenotypes and the overall reduction in infectious bites across the population compared to a susceptible population with no vector control treatment.

### **3.3.1 Assumptions**

The PM uses survival, infectious bite and reproduction data for each age class of each phenotype taken from the FCM, and therefore the assumptions underlying the FCM also apply to the PM.

The model uses the probability of survival to the start of each age class to calculate the initial population structure for susceptible phenotypes exposed to an intervention. By using survival, egg-laying and infectious bite values calculated as averages per cycle for mosquitoes alive at the start of each age cycle, this provides sufficient detail to generate the PM results. Although in some contexts mosquito population size has been observed fluctuating in waves, consistent with a sustained pattern of synchronised gonotrophic cycles, in general we must assume that the population at any point in time will comprise mosquitoes of all ages. The PM makes the simplifying assumption that eggs laid within a time period approximately equal to one gonotrophic cycle, centred around our modelled timepoints, can all be assumed to have the same mix of genotypes.

The model does not attempt to capture the effects of mutational processes or stochastic demographic effects on the origin and initial spread of very low numbers of resistance alleles; it is assumed that resistant alleles are already present at a low frequency in the population at the start of the analysis. Resistance involves a single gene and a simple dominant/recessive process. It is further assumed that the size and age structure of the population at the start of the PM analysis is that achieved after sustained use in a susceptible population of the insecticide being evaluated, that there is no immigration or emigration, and the proportion of each genotype in the new adults joining the population

matches that in the eggs from which they originate. Density dependence is assumed to occur at the mosquito larval stage, with the number of newly emerged adult mosquitoes joining the population in each modelled time period assumed to be constant, and to be the same as that for an untreated susceptible population.

The genetic make up of mating males in any cycle is the same as that calculated for newly-hatched mosquitoes in that cycle, and males of all genotypes are equally likely to mate successfully. Females are assumed to mate once only, in their first cycle, as is the norm [55,56]. The number of eggs produced per laying female is assumed to be unaffected by egg paternal genotype and all eggs laid are of equal quality and viability.

The proportion of infectious humans in the population is constant for all modelled time periods.

The resistant allele is assumed initially to be present in heterozygotes, forming a very small proportion of the population. Subsequent spread of the allele reflects the age-linked survival probabilities for susceptible and resistant mosquitoes in the presence of the insecticide, as well as the age-linked fecundity of each, as calculated in the FCM.

We use an implementation of the model in Excel [57], which analyses the changing status of the population for five thousand sequential discrete time periods, each equivalent to the length of one gonotrophic cycle.

### **3.3.2 Population Model Details**

The variables and parameters of the PM are detailed in Table 5.

The PM works in discrete time periods, each equivalent to the length of one gonotrophic cycle, with recruitment of newly emerged adult mosquitoes treated as occurring at the start of each time period. For each sequential time period, the proportion of the population comprised by each genotype in each age class,  $G_{g,n}$ , is calculated, reflecting the genotypes of new adult recruits and the survival of adults into each age class from the preceding period. This is then used to calculate  $R_n$ , the proportion of the population with a resistant



phenotype in period  $n$ , with  $R_n = G_{3,n} + G_{2,n}d$ .

Table 5 Variables and Parameters for Population Model

Variable or Parameter	Symbol	Comments & Constraints
Period number (periods over which the population is tracked)*	$n$	$0 < n$
Dominance of resistance allele	$d$	dominant $d = 1$ recessive $d = 0$
Genotype (normal allele $s$ , resistant allele $r$ )	$g$	$(s,s) \ g = 1$ $(s,r) \ g = 2$ $(r,r) \ g = 3$
Proportion of total population having genotype $g$ at start of period $n$	$G_{g,n}$	
Proportion of the population resistant at start of period $n$	$R_n$	
Average number of infectious bites per mosquito in population in period $n$	$M_n$	
Size of initial population (susceptibles in the presence of treatment) as proportion of base population (susceptibles without treatment)	$J$	value from FCM
Population size in period $n$ as proportion of initial population size	$W_n$	
Average infectious bites during one time period from an untreated population	$q$	value from FCM
Number of infectious bites from treated population during time period $n$ , expressed as a % of the number of infectious bites during one time period from a susceptible population without treatment,	$Q_n$	Chosen measure of control
Number of periods between egg-laying and adult emergence	$\Phi$	Input
Number of mosquito age classes included in analysis	$\lambda$	
Mosquito age (gonotrophic cycles)	$i$	$0 < i \leq \lambda$
Phenotype	$j$	susceptible $j = 1$ resistant $j = 2$
Probability of survival for mosquitoes with phenotype $j$ , to age $i+1$ from age $i$ ( $i \geq 1$ )	$S_{j,i}$	values from FCM
Mosquitoes with genotype $g$ at start of period $n$ as percentage of initial population	$Y_{gn}$	
Allele $a$ as proportion alleles contributed by male population in period $n$	$A_{a,n}$	$s \quad a = 1$ $r \quad a = 2$
Proportion of mosquitoes with genotype $g$ which survive from start of period $n$ to start of period $n+1$	$P_{g,n}$	
Proportion of mosquitoes with genotype $g$ which are age $i$ at start of period $n$	$C_{g,n,i}$	
Average number of eggs laid by females of phenotype $j$ , aged $i$	$F_{j,i}$	values from FCM
Total number of eggs with genotype $g$ laid in period $n$	$B_{g,n}$	
Proportion of all eggs laid in period $n$ having genotype $g$	$E_{g,n}$	
Proportion of all new adults having genotype $g$ at start of period $n$	$N_{g,n}$	$N_{2,1} = G_{2,1}$
Fitness factor for males with genotype $g$	$f_g$	
Average number of infectious bites per mosquito of phenotype $j$ aged $i$ in period $n$	$I_{j,i}$	values from FCM
New adults as % initial population	$K$	values from FCM
* As for the FCM, the duration of one gonotrophic cycle is used as a unit of time. For convenience we use 'cycles' to refer to mosquito age and 'periods' to refer to the sequential time periods for which values are calculated in the PM.		

Results from the FCM are used by the PM to calculate the average number of infectious bites per mosquito in the population during each time period. From this  $Q_n$ , the number

of infectious bites given by the population as a whole relative to those given by an untreated population, can be calculated for each time period as  $Q_n = \frac{M_n W_n J}{q}$ .

The proportion of the population having genotype  $g$  at the start of period  $n$  is calculated as the part of the population with that genotype at the beginning of the period, expressed as a proportion of the initial population size, divided by the total population size at the start of period  $n$ , likewise expressed as a proportion of the initial population size.

$$G_{g,n} = \frac{Y_{g,n}}{W_n}$$

The part of the population having genotype  $g$  at the start of period  $n$ ,  $Y_{g,n}$ , is calculated as the part of the population with that genotype at the beginning of the previous period multiplied by the proportion of such mosquitoes surviving to the end of the period, plus the new adults recruited at the start of period  $n$  with genotype  $g$ , all expressed as a proportion of the initial population size.  $Y_{2,1}$  and  $Y_{3,1}$  are input values,  $Y_{1,1} = 1 - Y_{2,1} - Y_{3,1}$  and

$$Y_{g,n} = Y_{g,n-1} P_{g,n-1} + K N_{g,n} \quad \text{with } n > 1$$

The three genotypes map to the two phenotypes, resistant,  $j=2$ , and susceptible,  $j=1$ , as follows;

$$\begin{aligned} g = 1 &\rightarrow j = 1 \\ g = 2 &\rightarrow j = 1 + d \\ g = 3 &\rightarrow j = 2 \end{aligned}$$

The proportion,  $P_{g,n}$ , of mosquitoes starting period  $n$  with genotype  $g$  which survive to the start of period  $n+1$ , is calculated as the sum of survival probabilities for mosquitoes with the phenotype generated by genotype  $g$  in each age group multiplied by the proportion of the mosquitoes with genotype  $g$  which are in that age group.

$$P_{g,n} = \sum_{i=1}^{\lambda-1} C_{g,n,i} S_{j,i}$$

$W_n$ , the total population size in period  $n$ , expressed as a proportion of the initial population size, is calculated as the total of the population sizes for each genotype, also expressed as a proportion of the initial population size.

$$W_n = \sum_{g=1}^3 Y_{g,n}$$

The proportion,  $N_{g,n}$ , of new adults joining the population with a given phenotype in a given period is equal to the proportion of the genotype among the eggs giving rise to the new adults.

$$N_{g,n} = E_{g,n-\Phi} \quad n > \Phi$$

$$N_{g,n} = E_{g,1} \quad n \leq \Phi$$

The average number of bites per mosquito in the population,  $M_n$ , is calculated as the sum of the totals for each genotype of the number of infectious bites per mosquito in each age category for mosquitoes with the applicable phenotype, multiplied by the proportion of mosquitoes with that genotype falling into each age category.

$$M_n = \sum_{g=1}^3 \left( G_{g,n} \sum_{i=1}^{\lambda} C_{g,n,i} I_{j,i} \right)$$

The PM starts with an age-structure for each genotype sub-population within the whole population,  $C_{g,1,i}$ , reflecting the survival values generated in the FCM for mosquitoes with that genotype subject to the appropriate treatment regime, including any relevant cost-of-resistance parameters.

In subsequent periods, the proportion of genotype  $g$  mosquitoes in age cycle 1 is calculated as the proportion of new adults with genotype  $g$  multiplied by the number of new adults

expressed as a proportion of the initial population size, divided by the total mosquitoes with genotype  $g$  at the start of period  $n$ , also expressed as a proportion of the initial population size

$$C_{g,n,1} = \frac{N_{g,n}K}{N_{g,n}K + Y_{g,n-1}P_{g,n-1}} \quad n > 1$$

For older age classes, the proportion of genotype  $g$  mosquitoes in each age cycle,  $C_{g,n,i}$  is calculated as the proportion of mosquitoes with genotype  $g$  falling into the preceding age category in period  $n-1$ , multiplied by the relevant survival probability, divided by the total mosquitoes with genotype  $g$  at the start of period  $n$

$$C_{g,n,i} = \frac{Y_{g,n-1}C_{g,n-1,i-1}S_{j,i-1}}{N_{g,n}K + Y_{g,n-1}P_{g,n-1}} \quad 1 < i \quad n > 1$$

The proportion  $E_{g,n}$ , of eggs produced in period  $n$  which have genotype  $g$ , is calculated as the number of eggs produced in period  $n$  with genotype  $g$ , divided by the total number of eggs produced in period  $n$ .

$$E_{g,n} = \frac{B_{g,n}}{B_{1,n} + B_{2,n} + B_{3,n}}$$

The numbers of eggs produced with each of the three possible genotypes,  $B_{g,n}$ , are calculated using the appropriate number of eggs per female for relevant phenotypes and ages, using the appropriate proportion of each genotype in the population to calculate the relevant allele contribution from the female population, multiplied by allele proportions appropriate to the male population in the period of mating for each female age class.

$$B_{1,n} = \sum_{i=1}^{\lambda} \left( (F_{1,i}C_{1,n,i}G_{1,n} + 0.5F_{1+d,i}C_{2,n,i}G_{2,n})A_{1,n-i+1} \right)$$

$$B_{2,n} = \sum_{i=1}^{\lambda} \left( F_{1,i} C_{1,n,i} G_{1,n} A_{2,n+1-i} + 0.5 F_{1+d,i} C_{2,n,i} G_{2,n} + F_{2,i} C_{3,n,i} G_{3,n} A_{1,n+1-i} \right)$$

$$B_{3,n} = \sum_{i=1}^{\lambda} \left( (F_{2,i} C_{3,n,i} G_{3,n} + 0.5 F_{1+d,i} C_{2,n,i} G_{2,n}) A_{2,n+1-i} \right)$$

The proportions,  $A_{1,n}$  and  $A_{2,n}$  of susceptible and resistant alleles available from the male population in period  $n$  is calculated based on the proportion of newly hatched males with each genotype, and any relative fitness adjustments applied to different phenotypes. Male genotypes for matings prior to the start of the modelled time period are assumed to be consistent with those in the population in the first modelled period, so  $A_{j,n} = A_{j,1} \quad n < 1$

$$A_{1,n} = (0.5 f_2 N_{2,n} + f_1 N_{1,n}) / (f_1 N_{1,n} + f_2 N_{2,n} + f_3 N_{3,n})$$

$$A_{2,n} = (0.5 f_2 N_{2,n} + f_3 N_{3,n}) / (f_1 N_{1,n} + f_2 N_{2,n} + f_3 N_{3,n})$$

### 3.4 Parameter Values for LLA Analysis

The formulation of key results in comparative terms reduces sensitivity of conclusions to specific parameter values. Sensitivity analysis (see section 3.6) confirms that variation in specific, non-intervention-related parameter values changes quantitative results but does not change our qualitative results and conclusions.

**Table 6 Values used in the FCM for this analysis**

Variable or Parameter	Symbol	Value	units
Background instantaneous mortality rate for mosquito age $i$	$r_{B,i}$	11.75% <sup>1</sup>	per day
Length of gonotrophic cycle	$w$	2.85 <sup>1</sup>	days
Time spent host searching and feeding during a cycle	$b$	1.26 <sup>5</sup>	days
Time spent finding oviposition site and laying during a cycle	$\phi$	1.26 <sup>5</sup>	days
Length of resting period (days)	$\eta$	0.32 <sup>5</sup>	days
Proportion human population infectious for malaria <sup>4</sup>	$p$	4.28% <sup>1</sup>	
Probability attacks non-human host	$H$	0.17 <sup>1</sup>	
Probability killed when attacking host before biting	$a_1$	.05 <sup>6</sup>	
Probability killed when attacking host after biting (excluding mortality from insecticide treatments)	$a_2$	.05 <sup>6</sup>	
Probability becomes infected with malaria when biting infectious human host <sup>4</sup>	$M$	1	

Variable or Parameter	Symbol	Value	units
Number of eggs laid per successfully laying mosquito per cycle	$L$	$100^2$	eggs
Time, measured in whole units equal to length of gonotrophic cycle, from infection of mosquito to cycle from which mosquito gives infectious bites	$D$	$3^3$ Based on 10.78 <sup>1</sup> days	cycles
Probability contacts and is killed by instant-kill insecticide when attacking human host, before biting, in cycle $i$	$k_{1,i,m}$	0 for cases not assessing use of CIKI or for ages below effective age of ADI Otherwise 0.8	
Number of age classes included in analysis	$\lambda$	10	cycles

1. Based on data from four geographic locations [51].  
 2. Since we are only interested in comparative values, the absolute value for the number of eggs per lay is immaterial, 100 has been used as a convenient normalised value.  
 3. The number of cycles assumed for sporogonic development is calculated from the average number of days for sporogonic development and the average number of days per gonotrophic cycle, rounded down to give a whole number of cycles. This is a conservative assumption with respect to the amount of EIR reduction calculated for given LLA parameters.  
 4. The data set used provides a total probability of acquiring a malaria infection when biting a human host. This has been used as the value for parameter  $p$ , with  $M=1.00$ , to give the appropriate combined probability,  $Mp$   
 5. Assumes c.11.1% of every cycle is spent resting (8 hours in a 72 hour cycle), with the rest of the gonotrophic cycle divided equally between laying and feeding  
 6. Estimated 10% mortality per feeding attempt [58], divided equally between pre- and post-bite.

Because the purpose of this analysis is to ‘test’ the potential of LLAs, where a choice arises, our intention has been to use conservative assumptions which will tend to understate rather than overstate the benefits (reduction in infectious bites and resistance management) of LLAs, so that any results generated in support of LLAs can be viewed as robust.

**Table 7 Values used in the PM for this analysis**  
 Time periods are equal in length to gonotrophic cycles, but we here use cycles to refer to units of mosquito age and periods to refer to units of time.

Variable or Parameter	Symbol	Value
Proportion of total population having genotype $g$ at start of period 1	$G_{g,1}$	$G_{1,1} = 1 - G_{2,1}$ $G_{2,1} = 10^{-9}$ $G_{3,1} = 0$
Dominance of resistance allele ( $0$ =recessive, $1$ =dominant)	$d$	$d = 1$
Number of periods between egg-laying and adult emergence	$\Phi$	3
Fitness factor for males with genotype $g$	$f_g$	$f_1 = f_2 = f_3 = 1.00$
<i>All other input values use results calculated by the FCM</i>		

### 3.5 Results

Using the FCM we calculate survival, egg-laying and infectious bite values for each age class for mosquitoes experiencing no intervention, a conventional instant-kill

intervention, and age-dependent instant-kill interventions effective in mosquitoes aged at least 2, 3, 4, 5 or 6 cycles old, assuming that all interventions are applied at 80% coverage using a pre-bite delivery method. Given our assumption of constant recruitment to age class 1, i.e. the number of new adults joining the population per unit of time is constant through time and for all interventions, the values we calculate per lifetime also give proportionate population-level values per unit of time. For example, the probability of mosquitoes surviving to the start of age class 3 under a given intervention, as a proportion of the total probabilities of survival to the start of all age classes, is also the proportion of mosquitoes joining that age class in the population as a whole.

From Table 8 it can be seen that the probability of survival through any given age class is reduced from 65% to 22% for ages affected by an insecticide. The way in which this changes population structure is summarised in Table 9.

**Table 8 : Summary of FCM survival results by age cycle**

Percentage probability of survival to the start and end of each age cycle, and the probability of surviving through each age cycle for mosquitoes in populations subject to no public health intervention, to a conventional instant-kill intervention, or to one of five age dependent insecticides.

Survival	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8	Cycle 9	Cycle10
No Intervention										
Survival to start of cycle	100%	64.6%	41.7%	26.9%	17.4%	11.2%	7.2%	4.7%	3.0%	2.0%
Survival to end of cycle	64.6%	41.7%	26.9%	17.4%	11.2%	7.2%	4.7%	3.0%	2.0%	1.3%
Survival through cycle	64.6%	64.6%	64.6%	64.6%	64.6%	64.6%	64.6%	64.6%	64.6%	64.6%
CIK										
Survival to start of cycle	100%	21.7%	4.7%	1.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%
Survival to end of cycle	21.7%	4.7%	1.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Survival through cycle	21.7%	21.7%	21.7%	21.7%	21.7%	21.7%	21.7%	21.7%	21.7%	21.7%
2-Cycle ADI										
Survival to start of cycle	100%	64.6%	14.0%	3.0%	0.7%	0.1%	0.0%	0.0%	0.0%	0.0%
Survival to end of cycle	64.6%	14.0%	3.0%	0.7%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
Survival through cycle	64.6%	21.7%	21.7%	21.7%	21.7%	21.7%	21.7%	21.7%	21.7%	21.7%
3-Cycle ADI										
Survival to start of cycle	100%	64.6%	41.7%	9.0%	2.0%	0.4%	0.1%	0.0%	0.0%	0.0%
Survival to end of cycle	64.6%	41.7%	9.0%	2.0%	0.4%	0.1%	0.0%	0.0%	0.0%	0.0%
Survival through cycle	64.6%	64.6%	21.7%	21.7%	21.7%	21.7%	21.7%	21.7%	21.7%	21.7%
4-Cycle ADI										
Survival to start of cycle	100%	64.6%	41.7%	26.9%	5.8%	1.3%	0.3%	0.1%	0.0%	0.0%
Survival to end of cycle	64.6%	41.7%	26.9%	5.8%	1.3%	0.3%	0.1%	0.0%	0.0%	0.0%
Survival through cycle	64.6%	64.6%	64.6%	21.7%	21.7%	21.7%	21.7%	21.7%	21.7%	21.7%
5 cycle ADI										
Survival to start of cycle	100%	64.6%	41.7%	26.9%	17.4%	3.8%	0.8%	0.2%	0.0%	0.0%
Survival to end of cycle	64.6%	41.7%	26.9%	17.4%	3.8%	0.8%	0.2%	0.0%	0.0%	0.0%
Survival through cycle	64.6%	64.6%	64.6%	64.6%	21.7%	21.7%	21.7%	21.7%	21.7%	21.7%
6 cycle ADI										
Survival to start of cycle	100%	64.6%	41.7%	26.9%	17.4%	11.2%	2.4%	0.5%	0.1%	0.0%
Survival to end of cycle	64.6%	41.7%	26.9%	17.4%	11.2%	2.4%	0.5%	0.1%	0.0%	0.0%
Survival through cycle	64.6%	64.6%	64.6%	64.6%	64.6%	21.7%	21.7%	21.7%	21.7%	21.7%

**Table 9 :Population Profile**

Population structure of mosquitoes at start of an age class. Percentage of total commencing each age class.

Population Profile <sup>1</sup>	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8	Cycle 9	Cycle10
No Intervention	36%	23%	15%	10%	6%	4%	3%	2%	1%	1%
CIK Insecticide	78%	17%	4%	1%	0%	0%	0%	0%	0%	0%
2-Cycle ADI	55%	35%	8%	2%	0%	0%	0%	0%	0%	0%
3-Cycle ADI	46%	30%	19%	4%	1%	0%	0%	0%	0%	0%
4-Cycle ADI	42%	27%	17%	11%	2%	1%	0%	0%	0%	0%
5-Cycle ADI	39%	25%	16%	11%	7%	1%	0%	0%	0%	0%
6-Cycle ADI	38%	24%	16%	10%	7%	4%	1%	0%	0%	0%

*1. Assuming that the number of new adults joining the population per unit of time remains constant through time and for all interventions*

Infectious bite values are affected by survival within, as well as between age classes (Table 10). No infectious bites are given by mosquitoes younger than the malaria development



period, in this case, 3 cycles. Thereafter the proportion of infectious bites per mosquito in a given age class increases with age, as previously acquired malaria infections mature and reach an infectious stage. For the total number of infectious bites given by mosquitoes in each age class this effect is counterbalanced, and ultimately outweighed, by the decreasing numbers of mosquitoes surviving into each age class. Thus, in the absence of an intervention, age class 5 generates the most infectious bites per cycle, more than age class 4, since more malaria infections have matured by cycle 5, more than age class 6, since mortality outweighs the increase in infectiousness between cycles 5 and 6. With interventions the picture is more complex, and for all but the 6-cycle effective age ADI, mortality effects outweigh the increasing proportion of mature infections, and the number of infectious bites per age class decreases from the 1<sup>st</sup> infectious cycle.

**Table 10 Summary of FCM infectious bite results by age cycle**  
Average number of infectious bites (× 1,000) given during a mosquito lifetime, broken down by age class, and the average number of infectious bites given by mosquitoes surviving to the start of each age class, whilst in that age class.

Infectious Bites (x 1,000)	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8	Cycle 9	Cycle10	Total per lifetime
<b>No Intervention</b>											
During cycle	0.00	0.00	0.00	6.49	8.23	7.83	6.63	5.26	4.00	2.96	<b>41.41</b>
Per mosquito starting cycle	0.00	0.00	0.00	24.12	47.38	69.82	91.46	112.33	132.46	151.88	
<b>CIK Insecticide</b>											
During cycle	0.00	0.00	0.00	0.03	0.01	0.00	0.00	0.00	0.00	0.00	<b>0.05</b>
Per mosquito starting cycle	0.00	0.00	0.00	2.87	5.68	8.43	11.13	13.76	16.34	18.87	
<b>2-Cycle ADI</b>											
During cycle	0.00	0.00	0.00	0.15	0.05	0.01	0.00	0.00	0.00	0.00	<b>0.22</b>
Per mosquito starting cycle	0.00	0.00	0.00	4.82	7.59	10.30	12.96	15.55	18.10	20.59	
<b>3-Cycle ADI</b>											
During cycle	0.00	0.00	0.00	0.44	0.19	0.05	0.01	0.00	0.00	0.00	<b>0.69</b>
Per mosquito starting cycle	0.00	0.00	0.00	4.82	9.48	12.15	14.76	17.32	19.83	22.28	
<b>4-Cycle ADI</b>											
During cycle	0.00	0.00	0.00	1.30	0.55	0.18	0.05	0.01	0.00	0.00	<b>2.09</b>
Per mosquito starting cycle	0.00	0.00	0.00	4.82	9.48	13.96	16.54	19.06	21.53	23.95	
<b>5 cycle ADI</b>											
During cycle	0.00	0.00	0.00	6.49	1.65	0.53	0.15	0.04	0.01	0.00	<b>8.86</b>
Per mosquito starting cycle	0.00	0.00	0.00	24.12	9.48	13.96	18.29	20.78	23.21	25.59	
<b>6 cycle ADI</b>											
During cycle	0.00	0.00	0.00	6.49	8.23	1.57	0.45	0.12	0.03	0.01	<b>16.89</b>
Per mosquito starting cycle	0.00	0.00	0.00	24.12	47.38	13.96	18.29	22.47	24.86	27.21	

The relative number of eggs laid per lifetime is our measure of fitness. As can be seen from Table 11, even the 2-cycle ADI offers a substantial fitness benefit compared to a CIKI, and this increases with the effective ages of ADIs.

Comparative fitness values and EIR reduction values are summarised in Table 12. The selection coefficient indicates the strength of selection in favour of resistance, so high selection coefficients indicate a high strength of selection for resistance. Predictably, whilst resistance management benefits increase with increasing ADI effective ages, the transmission reduction opportunities indicated by the reduction in EIR are maximised with lower ADI effective ages.

**Table 11 Summary of FCM reproduction results by age cycle**  
Average number of eggs laid during a mosquito lifetime, broken down by age class, and the average number of eggs laid by mosquitoes surviving to the start of each age class, whilst in that age class.

Reproduction <sup>1</sup>	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8	Cycle 9	Cycle10	Total per lifetime
<b>No Intervention</b>											
Average in cycle	64.6	41.7	26.9	17.4	11.2	7.2	4.7	3.0	2.0	1.3	179.93
per mosquito starting cycle	64.6	64.6	64.6	64.6	64.6	64.6	64.6	64.6	64.6	64.6	
number in cycle as % total	36%	23%	15%	10%	6%	4%	3%	2%	1%	1%	
<b>CIK Insecticide</b>											
Average in cycle	21.7	4.7	1.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	27.71
per mosquito starting cycle	21.7	21.7	21.7	21.7	21.7	21.7	21.7	21.7	21.7	21.7	
<b>2-Cycle ADI</b>											
Average in cycle	64.6	14.0	3.0	0.7	0.1	0.0	0.0	0.0	0.0	0.0	82.46
per mosquito starting cycle	64.6	21.7	21.7	21.7	21.7	21.7	21.7	21.7	21.7	21.7	
<b>3-Cycle ADI</b>											
Average in cycle	64.6	41.7	9.0	2.0	0.4	0.1	0.0	0.0	0.0	0.0	117.81
per mosquito starting cycle	64.6	64.6	21.7	21.7	21.7	21.7	21.7	21.7	21.7	21.7	
<b>4-Cycle ADI</b>											
Average in cycle	64.6	41.7	26.9	5.8	1.3	0.3	0.1	0.0	0.0	0.0	140.63
per mosquito starting cycle	64.6	64.6	64.6	21.7	21.7	21.7	21.7	21.7	21.7	21.7	
<b>5 cycle ADI</b>											
Average in cycle	64.6	41.7	26.9	17.4	3.8	0.8	0.2	0.0	0.0	0.0	155.37
per mosquito starting cycle	64.6	64.6	64.6	64.6	21.7	21.7	21.7	21.7	21.7	21.7	
<b>6 cycle ADI</b>											
Average in cycle	64.6	41.7	26.9	17.4	11.2	2.4	0.5	0.1	0.0	0.0	164.88
per mosquito starting cycle	64.6	64.6	64.6	64.6	64.6	21.7	21.7	21.7	21.7	21.7	

1. Calculated using a normalised value of 100 eggs per lay for mosquitoes of all ages and status

The PM allows the relative fitness values calculated in the FCM to be translated into comparative times to loss of efficacy as resistance spreads. From Figure 18 it can be seen that ADIs with higher effective ages offer lower reductions in EIR before the spread of resistance, but that these reductions are maintained for longer, as spreading resistance erodes the population-level EIR reductions achieved by CIKIs and earlier effective age ADIs. Even with a 2-cycle ADI resistance takes between 2 and 3 times as long to spread as with a CIKI.

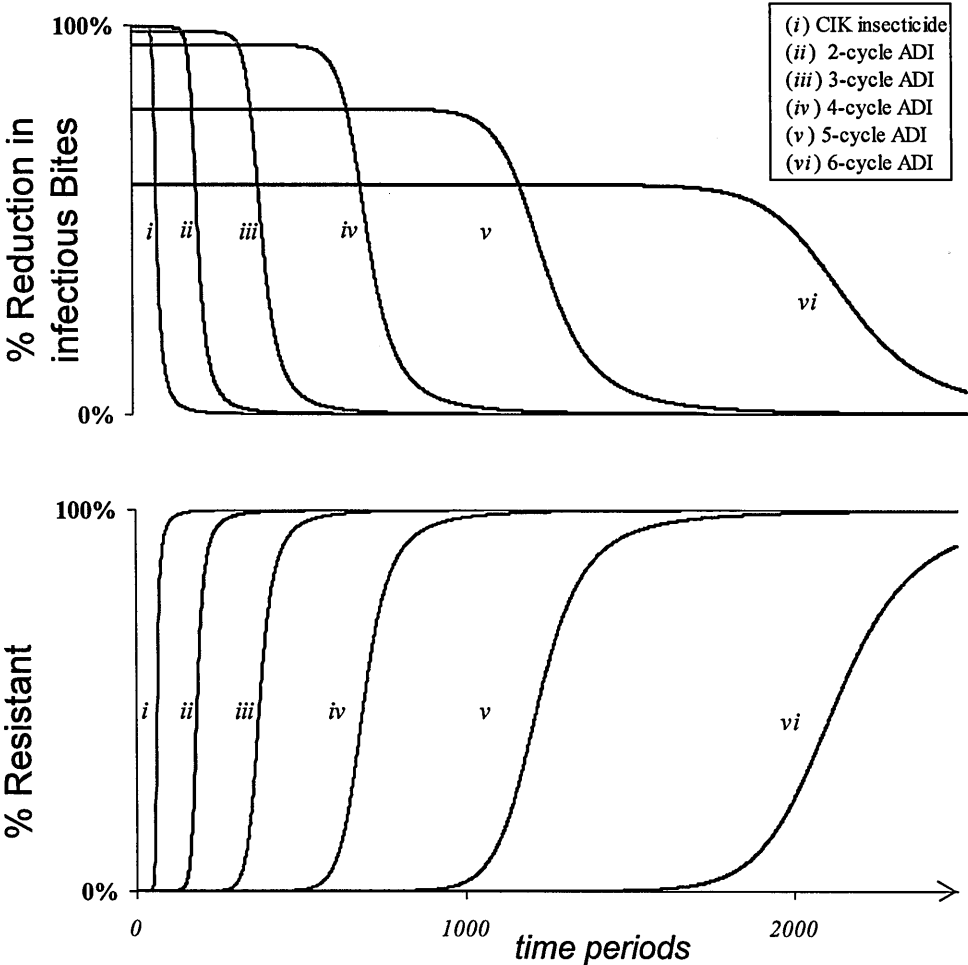
**Table 12 : Selection coefficient and RAIB values for CIKI and ADIs**

Summary of results for conventional and age-dependent insecticides. The selection coefficients are the proportionate reduction in lifetime reproductive success for mosquitoes susceptible to each intervention. Higher selection coefficients indicate stronger selection pressure in favour of resistance. The reduction in EIR is the proportionate reduction in population-level infectious bites.

	Selection Co-efficient	Relative fitness of resistant mosquitoes when intervention applied	Reduction in EIR
Conventional Instant-Kill Insecticide	0.85	6.5	99.9%
2-Cycle ADI	0.54	2.2	99.5%
3-Cycle ADI	0.35	1.5	98.3%
4-Cycle ADI	0.22	1.3	95.0%
5-Cycle ADI	0.14	1.2	78.6%
6-Cycle ADI	0.08	1.1	59.2%

**Figure 18: Results from the PM showing the spread of resistance over time, and the consequent loss of reduction in EIR.**

Changing values over time for resistant phenotypes as a proportion of the population (bottom panel) and consequent loss of reduction in population level infectious bites (top panel) for a CIKI and ADIS with effective ages of 2, 3, 4, 5 and 6 cycles. All insecticides assumed delivered pre-bite with 80% coverage.



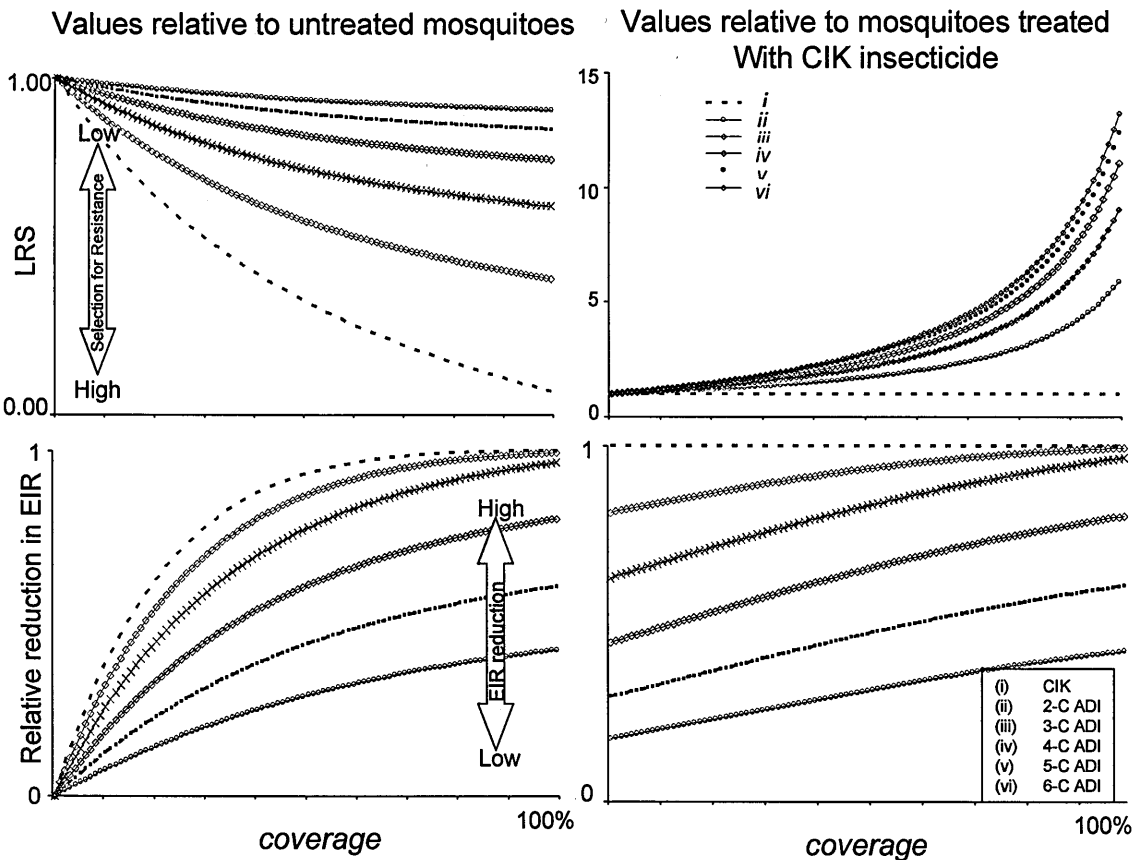
### 3.5.1 Coverage

The probability that a mosquito contacts and is affected (killed or contaminated) by an insecticide, which we here refer to as ‘coverage’, affects EIR reduction and selection coefficient values for all insecticide types. However, as can be seen from Figure 19 the magnitude of these effects varies for the CIKIs and ADIs we evaluated. It can be seen (Figure 19 left hand panels) that coverage levels have a proportionately greater impact on both LRS and reduction in EIR for CIKIs and lower effective age ADIs. From the right hand panels of Figure 19, which show LRS and reduction in EIR for all insecticides as proportions of those for a CIKI, it can be seen that, when compared to the best existing alternative, the relative performance of ADIs is maximised for both transmission control and resistance management, at high coverage values.

Comparison of Figure 18 and Figure 20 shows the improvement in time to speed of spread of resistance for all insecticides assuming 40% rather than 80% coverage. However, the relative time for spread of resistance to ADIs is reduced compared to that for CIKIs and in all cases, initial reduction in EIR is reduced. This is also illustrated in the plots in Figure 21, which shows the speed of spread of resistance and loss in EIR reduction for a 4-cycle effective age ADI assuming 80%, 60%, 40% and 20% coverage.

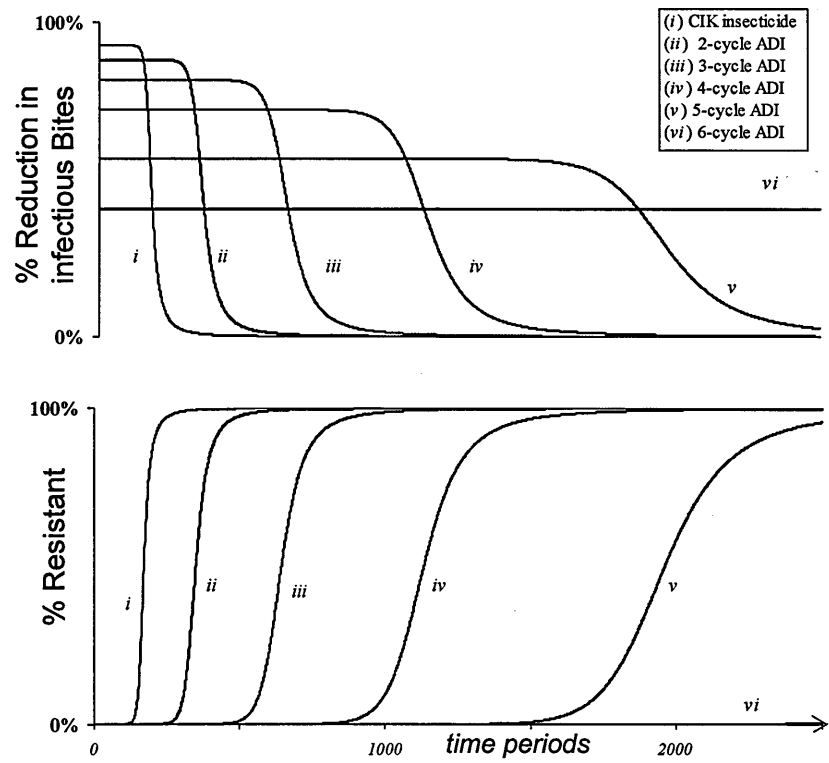
**Figure 19 : Comparison of conventional instant-kill chemical insecticide and ADIs across a range of coverage values**

Lifetime reproductive success with interventions as a proportion of LRS for untreated mosquitoes (top left panel) and as a proportion of LRS for mosquitoes treated with an instant-kill insecticide (top right panel). Reduction in average infectious bites per mosquito lifetime with interventions, compared to the value for untreated mosquitoes (bottom left panel), 0 = no reduction in infectious bites, 1.00 = no infectious bites. Reduction in infectious bites with interventions vs untreated mosquitoes, compared to the reduction achieved using a conventional instant kill insecticide (bottom right panel), 1.00 means a reduction equal to that achieved by instant-kill insecticide.



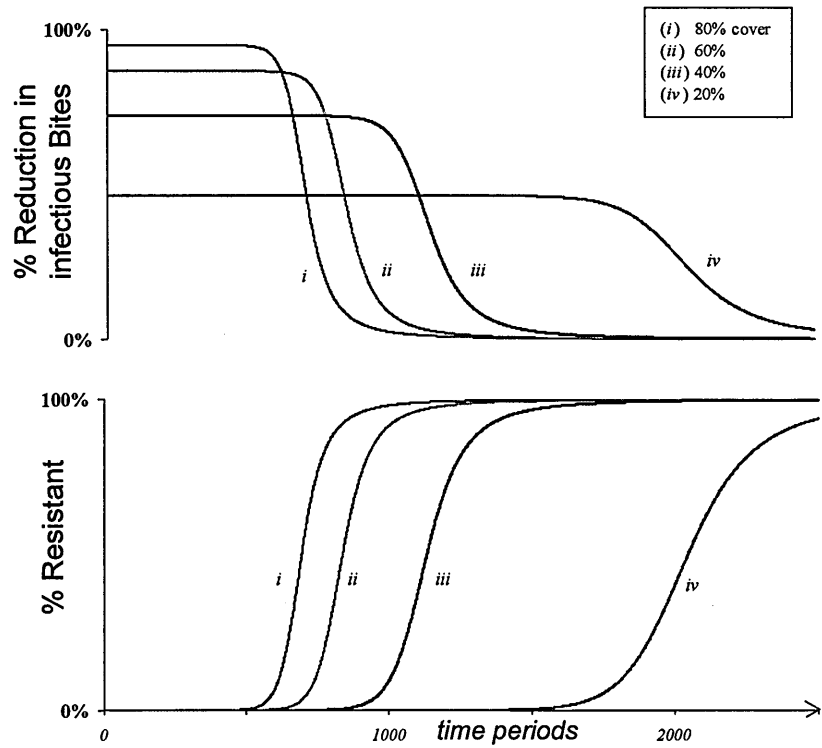
**Figure 20** Results from the PM showing the spread of resistance over time, and the consequent loss of reduction in EIR, with 40% insecticide coverage.

Changing values over time for resistant phenotypes as a proportion of the population (bottom panel) and consequent loss of reduction in population level infectious bites (top panel) for a CIKI and ADIS with effective ages of 2, 3, 4, 5 and 6 cycles assumed delivered pre-bite with 40% coverage.



**Figure 21 :** Results from the PM showing the spread of resistance over time, and the consequent loss of reduction in EIR, for a range of insecticide coverage values.

Changing values over time for resistant phenotypes as a proportion of the population (bottom panel) and consequent loss of reduction in population level infectious bites (top panel) for a 4-cycle ADI, assumed delivered pre-bite with 80%, 60%, 40% and 20% coverage.



### 3.5.2 Delivery method for CIKIs and ADIs

The two most commonly adopted delivery methods for the control of adult mosquitoes are insecticide treated bed nets (ITNs) and indoor residual spraying (IRS). Both deliver insecticides to indoor-biting mosquitoes immediately prior to a feeding attempt (ITNs) or shortly after feeding (ITNs and IRS). We here consider the significance of these two different delivery methods for LLA insecticides.

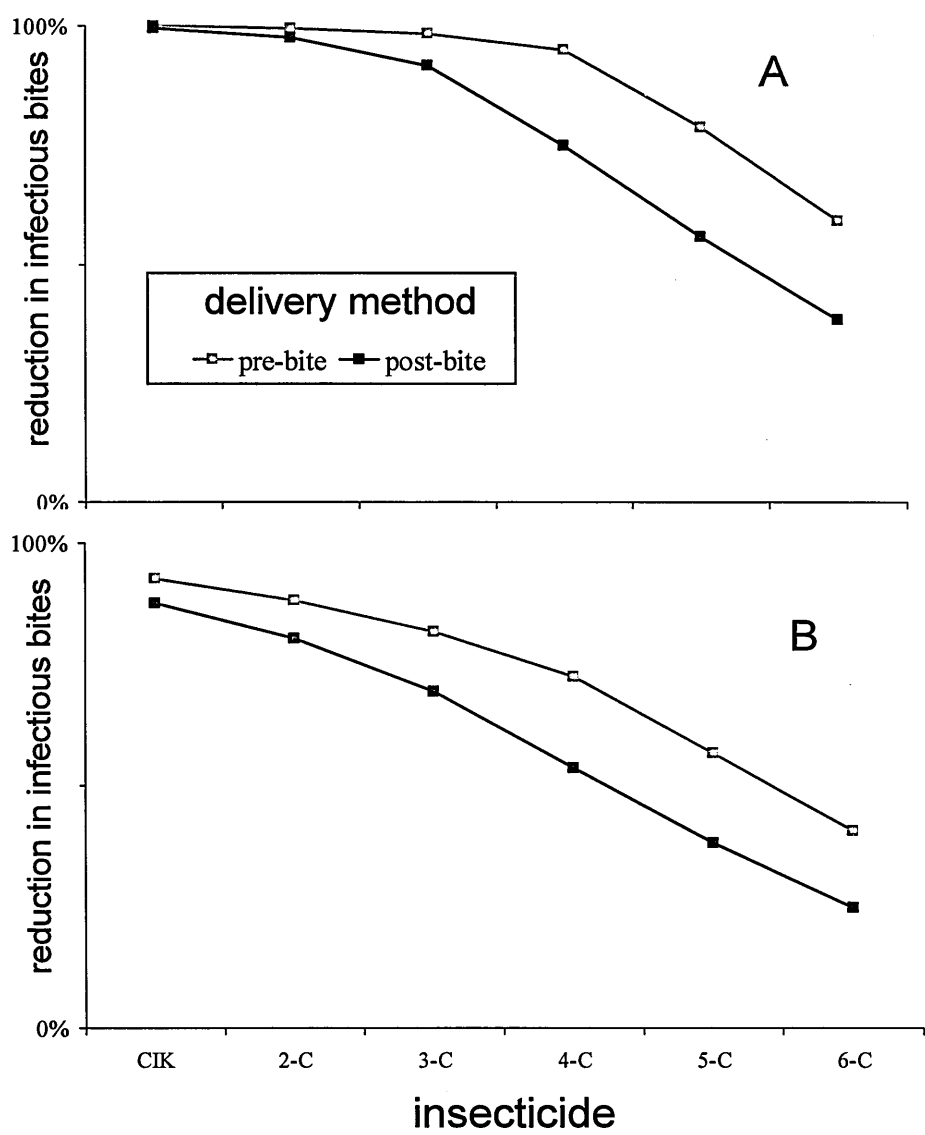
For instant-kill insecticides, CIKIs and ADIs, the difference between pre-bite or post-bite exposure does not affect the likelihood of surviving to the end of an age class, nor of surviving to oviposit. It is only the probability of biting in a given cycle which is affected by the choice between these two delivery methods. This in turn affects two key probabilities, the probability of a mosquito becoming infected with malaria, and the probability of a mosquito giving an infectious bite. A mosquito which dies immediately prior to biting, and one which survives to bite and acquires a new malaria infection, then dies immediately, have the same zero probability of giving an infectious bite, and so are identical for all our key metrics. This is not the case, however, for an infectious mosquito, which gives an infectious bite if killed post-bite, but is prevented from doing so if killed pre-bite.

For TDIs, which kill some time after contacting and contaminating a mosquito, the timing of mortality is not inevitably linked to whether initial contact occurs prior to or after feeding, being determined by the characteristics of the TDI after contact. We therefore explore the impact on RAIB of a post, rather than pre-bite delivery method for CIKIs and ADIs only.

It can be seen from Figure 22 that in all cases, use of a post-bite delivery method reduces the RAIB to a level below that available with a one cycle higher effective age ADI delivered pre-bite. From Figure 23 it can be seen that the difference between the two delivery methods is greater for higher effective ages, and for lower coverage values.

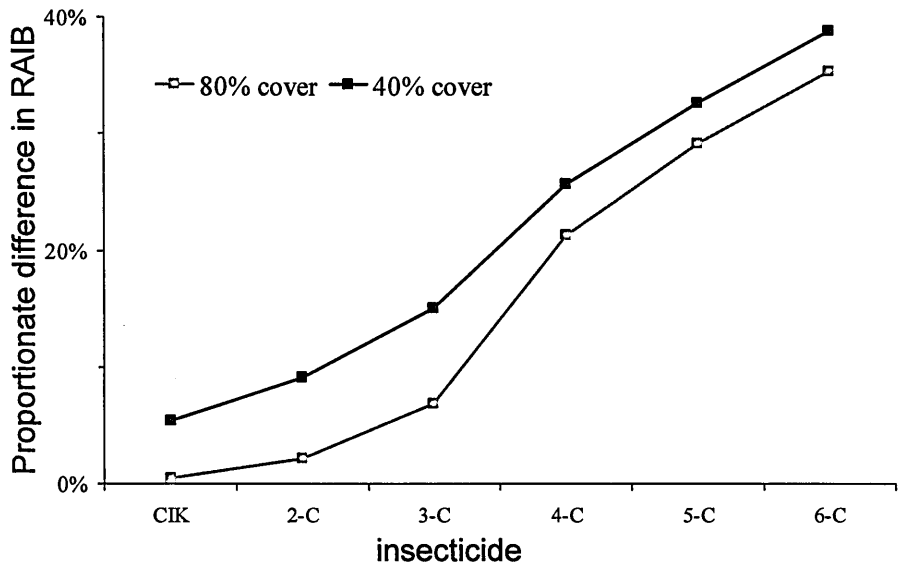
Since the LRS values are unaffected by this issue, the reduction in RAIB with post-bite delivery is not offset by any improvement in the LRS. Choice of delivery method may therefore be key to accessing the best possible combination of transmission control and resistance management using ADIs.

**Figure 22 : Comparison of pre-bite and post-bite delivery systems**  
Plotted values are the reduction in EIR for conventional and age-dependent instant-kill insecticides applied using delivery methods which result in contact either immediately before or immediately after biting a human host. ADI values are shown for ADIs which are effective for mosquitoes aged 2, 3, 4, 5 or 6 cycles or above. Panel A assumes 80%, and panel B 40% coverage. All results assume 3 cycles between infection and infectiousness of malaria in mosquito host.





**Figure 23 : Proportionate difference between pre- and post-bite delivery methods**  
 Plotted values are the proportionate difference in RAIB between pre and post-bite delivery systems, for conventional and age-dependent instant-kill insecticides at 80% or 40% coverage. ADI values are shown for ADIs which are effective for mosquitoes aged 2, 3, 4, 5 or 6 cycles or above. All results assume 3 cycles between infection and infectiousness of malaria in mosquito host.



### 3.5.3 Relationship between ADI effective age and malaria pre-patent period

It is clear from Figure 22 that, with a pre-bite delivery method, reduction in EIR falls away steeply for ADIs with effective ages of 4 cycles or more. In order to test how far this profile is dependent on the assumed period of malaria development in the mosquito host, we calculated results using our base assumptions, with pre-bite delivery for all insecticides at 80% coverage, assuming malaria pre-patent periods equivalent to 2, 3, 4, 5 and 6 gonotrophic cycles.

**Table 13 Summary of LRS and AIB values without interventions, relative to base case values, for various malaria development periods**

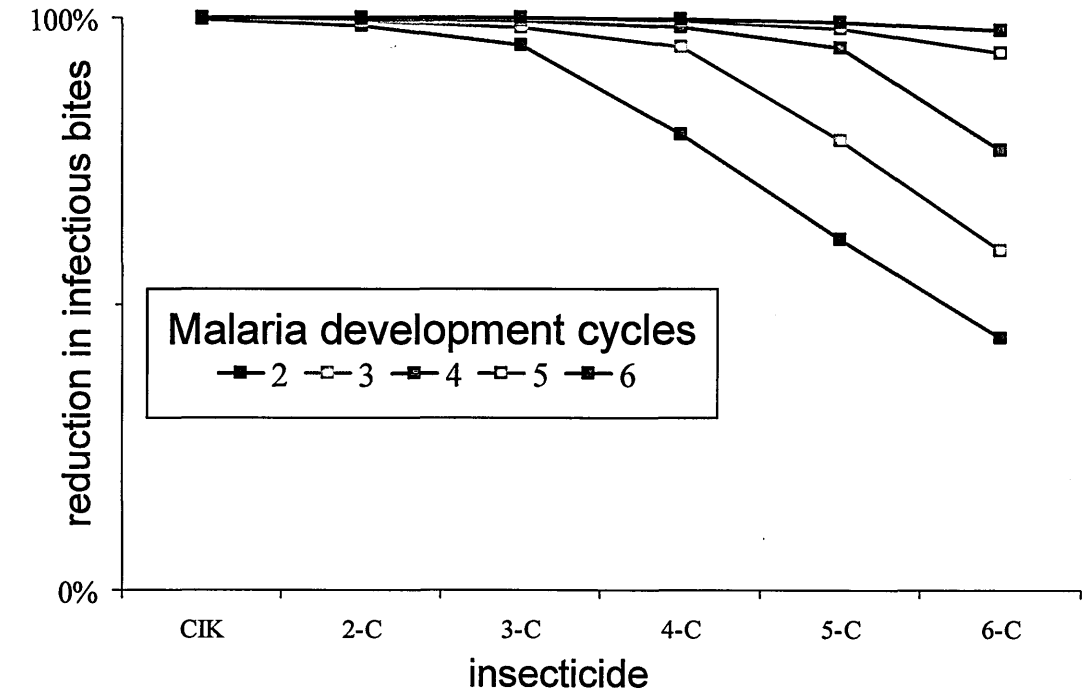
<i>Results for untreated populations</i>	<i>relative to base case values</i>	
	EIR	LRS
2-cycle malaria development period	1.63	1.00
4-cycle malaria development period	0.60	1.00
5-cycle malaria development period	0.35	1.00
6-cycle malaria development period	0.19	1.00

As can be seen from Table 13, the malaria development period has a substantial impact on the EIR in the absence of any intervention, relative to our base case assumption of 3 development cycles.

The results with interventions indicate a clear relationship between the malaria development period and the ADI effective ages with which the highest transmission control can be achieved (Figure 24). ADIs effective for ages up to 1 cycle more than the malaria pre-patent period offer similar levels of EIR reduction, with efficacy falling away steeply for effective ages above this. Within this group ADIs with effective ages 1 cycle greater than the malaria pre-patent period offer lower EIR reductions than ADIs with earlier effective ages, and ADIs with effective ages one cycle less than the malaria pre-patent period offer EIR reductions comparable to those for CIKs.

Assuming no impact from malaria infection on mosquito survival and fecundity, resistance management benefits are not affected by the malaria development period and so are maximised with higher ADI effective ages. For high coverage with pre-bite delivery methods, ADIs with effective ages equal to the malaria period of development  $\pm 1$  cycle therefore offer the best range of options, depending upon context, from which to select the optimum combination of transmission reduction and resistance management.

**Figure 24: Conventional and age-dependent instant-kill insecticide effectiveness for various malaria development periods**  
 Plotted values are the proportionate reduction in EIR for conventional and age dependent instant-kill insecticides assuming malaria development periods equivalent to 3, 4, 5 or 6 mosquito gonotrophic cycles. All results assume 80% coverage and a pre-bite delivery method

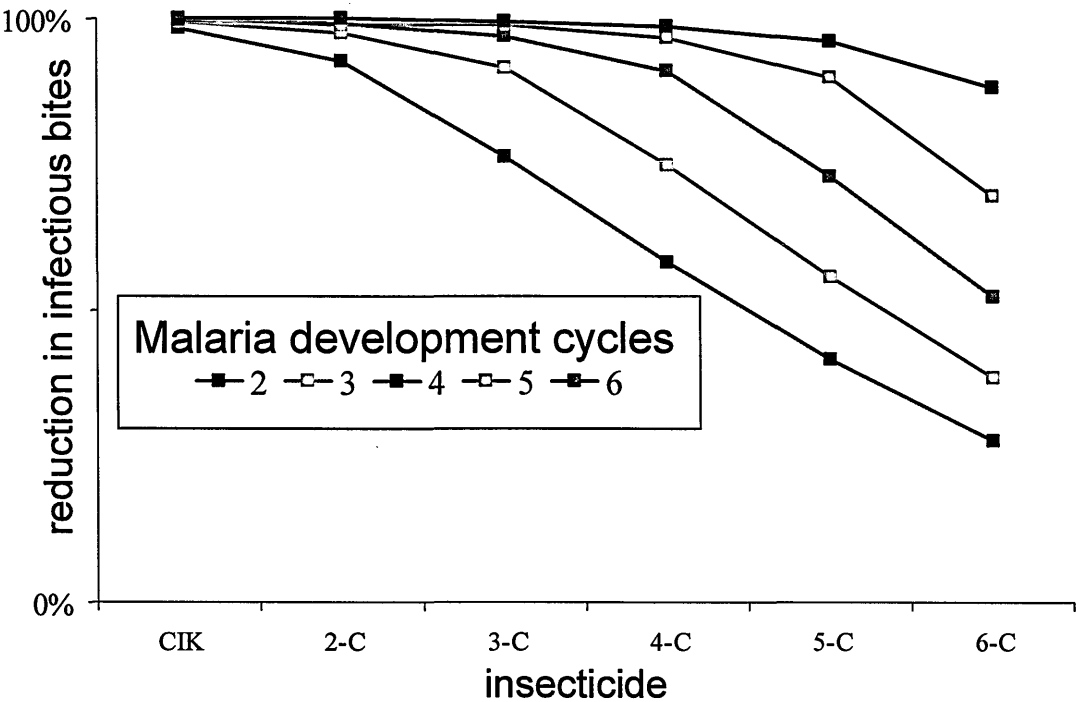


The same evaluation assuming a post-bite delivery system does not simply show a one-cycle reduction in the useful ADI effective ages, as might be expected. EIR reductions for ADIs with effective ages equal to or greater than the malaria development period, are consistently lower than those offered by ADIs with an effective age 1 cycle higher using a pre-bite delivery method (Figure 25). For a post-bite delivery method used with high coverage, the useful range of ADIs appears to be those with effective ages up to 1 cycle less than the malaria pre-patent period.

With low (40%) coverage the reduction in EIR is more sensitive to the speed of malaria development for CIKI and low effective-age ADIs (Figure 26). There is no clear dividing line between ADI effective ages offering reasonable and poor transmission control, and no clear relationship between malaria time to maturity and useful ADI effective age.

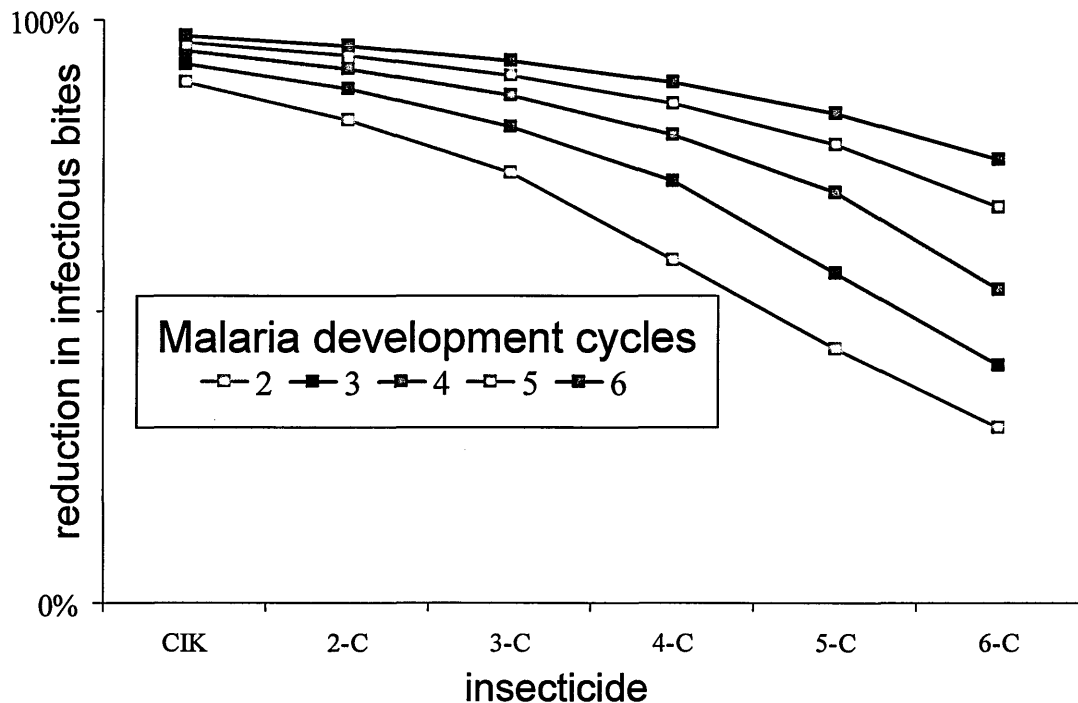
**Figure 25: Instant-kill insecticide effectiveness for various malaria development periods with post-bite delivery method**

Plotted values are the proportionate reduction in EIR for conventional and age dependent instant-kill insecticides assuming malaria development periods equivalent to 3, 4, 5 or 6 mosquito gonotrophic cycles. All results assume 80% coverage and a post-bite delivery method



**Figure 26 Instant-kill insecticide effectiveness for various malaria development periods with 40% coverage**

Plotted values are the proportionate reduction in EIR for conventional and age dependent instant-kill insecticides assuming malaria development periods equivalent to 3, 4, 5 or 6 mosquito gonotrophic cycles. All results assume 40% coverage and a pre-bite delivery method

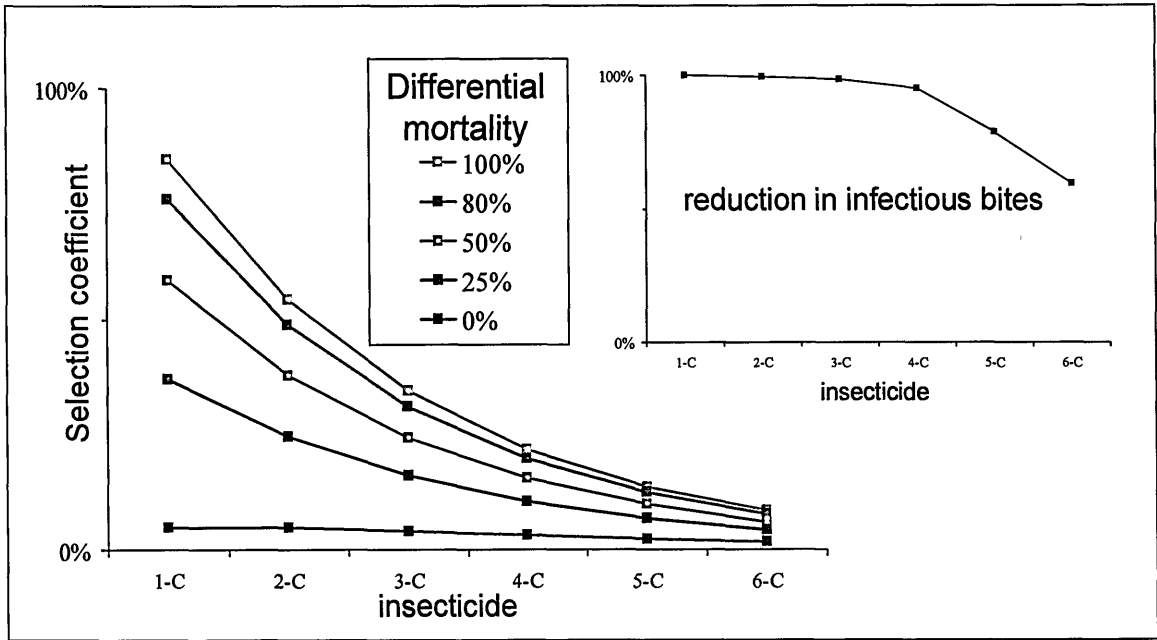


### 3.5.4 Differential insecticide mortality in mosquitoes with malaria infection

Since our purpose is to control malaria, not to control mosquitoes, interventions which preferentially target malaria-infected mosquitoes may offer the ultimate opportunity to achieve high transmission control with very low selection for resistance. From Figure 27 it can be seen that, with high coverage, insecticides which have a mortality differential between malaria-infected and uninfected mosquitoes offer substantially improved resistance management (lower selection coefficient, left hand panel) with no loss of transmission control (reduction in infectious bites, right hand panel). The differential mortality percentages are the mortality produced by an ADI in malaria-free mosquitoes as a percentage of that produced in malaria-infected mosquitoes. Malaria-infected mosquitoes experience the full mortality associated with each insecticide at 80% coverage.

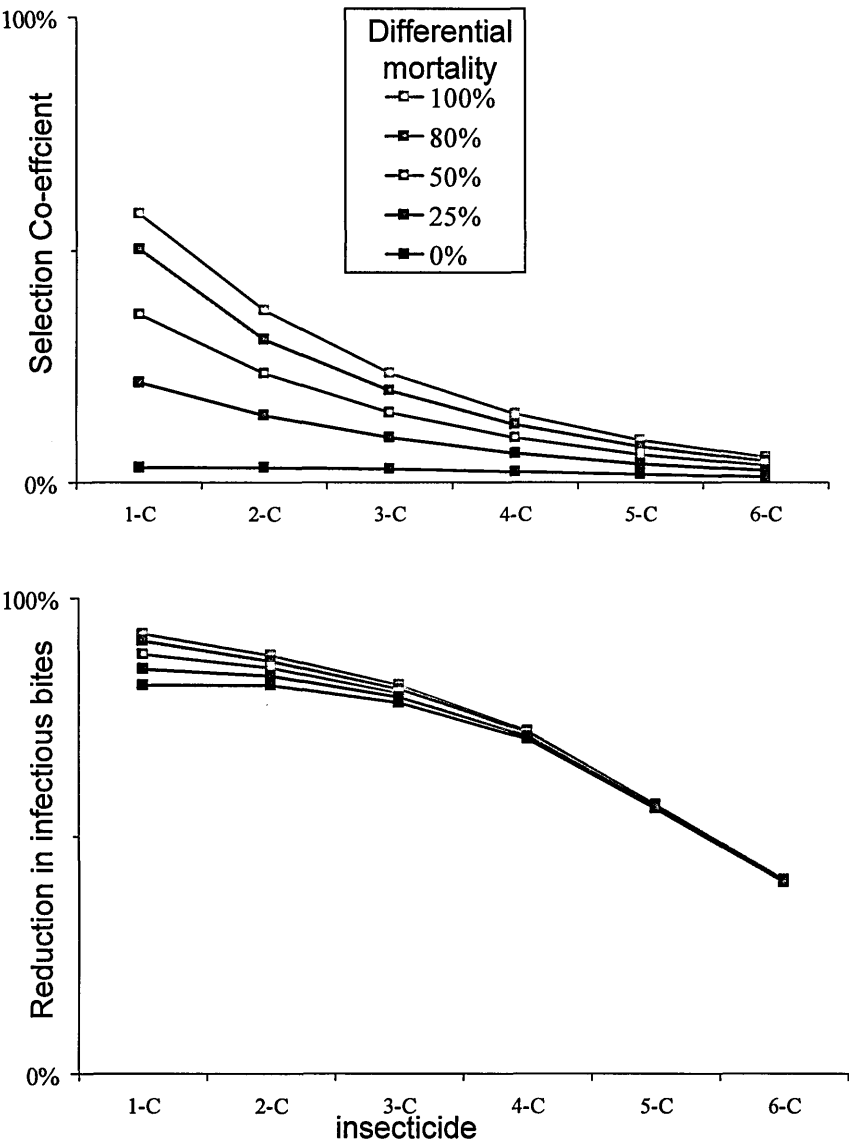
**Figure 27 Selection Coefficient for ADIs with differential mortality in malaria infected and uninfected mosquitoes.**

ADI mortality in malaria uninfected mosquitoes is the specified percentage of the mortality in malaria-infected mosquitoes. Inset shows the corresponding reductions in infectious bites for all differential mortality assumptions. All results assume 3-cycle malaria pre-patent period and 80% coverage.



A similar pattern is seen at 40% coverage (Figure 28) with regard to resistance management, however, there is also a cost in terms of lost EIR reduction, particularly for CIKI and early effective age ADIs. This is presumably a consequence of additional survivors amongst malaria uninfected mosquitoes which become infected and bite, an effect more significant for additional survivors in early age classes, and hence for CIKI and early effective age ADIs.

**Figure 28 Selection Coefficient and reduction in infectious bites for ADIs with differential mortality in malaria infected and uninfected mosquitoes at 40% coverage. ADI mortality in malaria uninfected mosquitoes is the specified percentage of the mortality in malaria-infected mosquitoes. Results assume 3-cycle malaria pre-patent period and 40% coverage.**



### 3.5.5 Costs of resistance

Complete evolution-proofing can be achieved if there are high enough costs of resistance (COR). The actual magnitude of the costs of insecticide resistance in *Anopheles* are unclear. A figure has, however, been calculated for the fitness cost of resistance in the non-malaria vector *Culex pipiens*. The fitness of *Culex pipiens* organophosphorous (OP) resistant homozygotes relative to susceptible homozygotes, following 40 years of OP insecticide spraying in Southern France, was calculated as between 0.63–0.72 [59,60]. We calculate that resistant mosquitoes are less fit than susceptibles for costs of

resistance  $\geq 3.43\%$  additional daily mortality. This gives a relative fitness value for resistant mosquitoes of 0.78, a lower COR than that observed in *Culex pipiens*, so we know that evolution proofing is possible with biologically plausible costs of resistance.

**Figure 29 Results from the PM showing the spread of resistance over time, and the consequent loss of reduction in EIR with various costs of resistance.**

Resistant phenotypes as a proportion of the population (bottom panel) and consequent loss of reduction in population level infectious bites (top panel) with range of assumed costs of resistance. Cost of resistance is applied as an addition to daily mortality rates at the percentages shown. All results are for a 4 cycle ADI at 80% coverage.

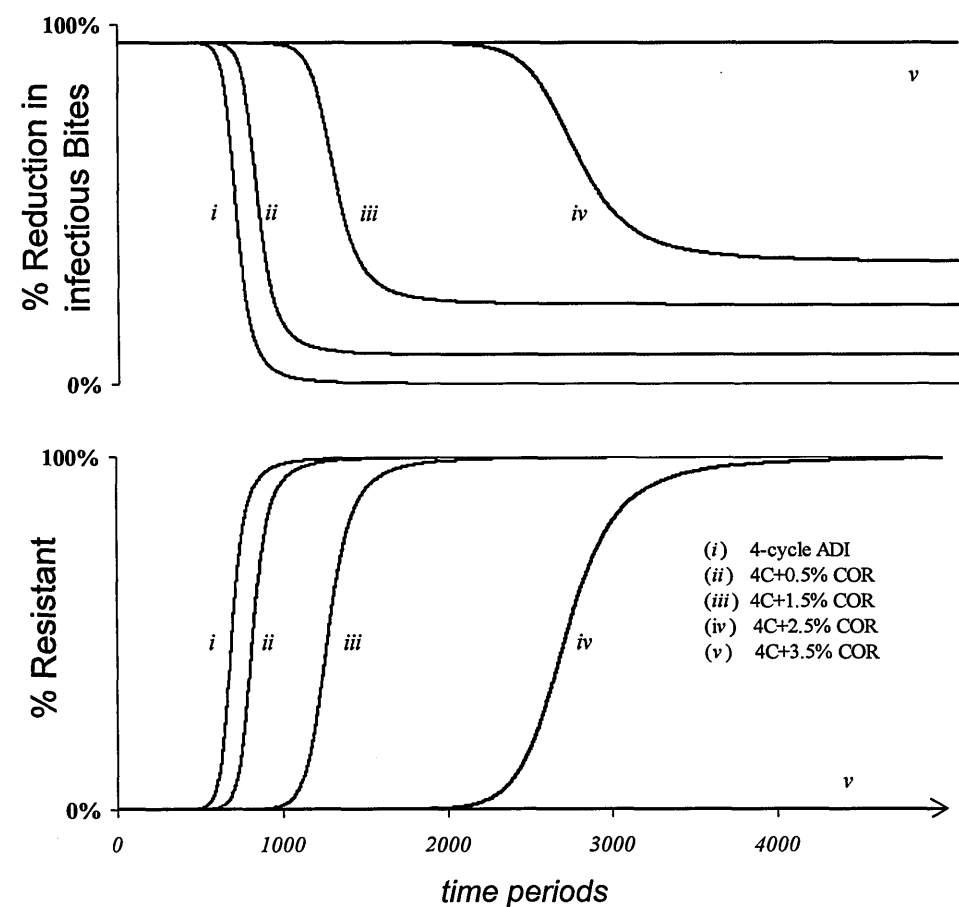


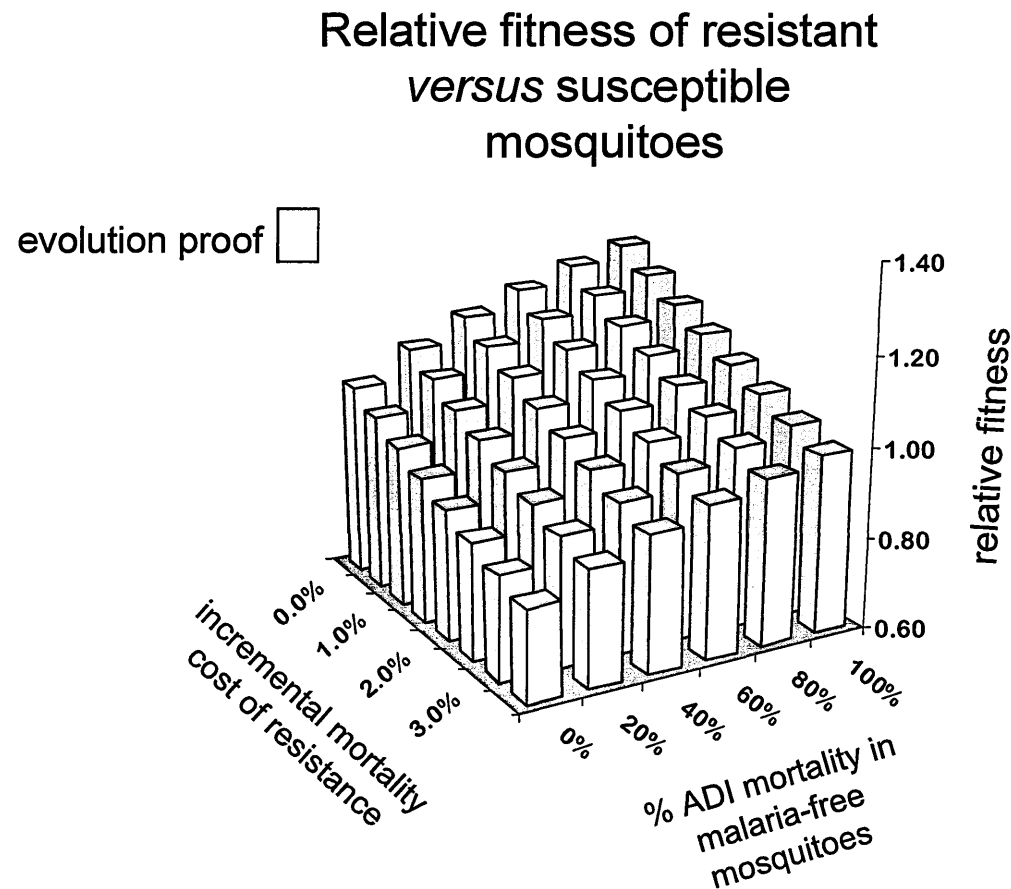
Figure 29 summarises results from the PM for a 4-cycle ADI at 80% pre-bite coverage with a range of COR. All plots have the same initial EIR reduction. It can be seen that increasing COR predictably extends the time taken for resistance to spread. For a COR of +3.0% daily mortality, even when the population is wholly comprised of resistant phenotypes, the increased mortality of resistant mosquitoes means that EIR reduction remains at around 30% compared to an untreated susceptible population. With 3.5% COR, resistance does not spread during the 5,000 modelled time periods. This is consistent with the <1.0 relative fitness calculated for resistants with this COR in the FCM. If COR is

realised as a direct fecundity reduction, with no additional mortality, a 22% reduction in fecundity produces evolution proofing for a 4-cycle effective age ADI, at 80% coverage delivered pre-bite, with our base case assumptions. With no survival costs, COR only affects the selection coefficient, transmission reductions are the same as those assuming no COR.

### 3.5.6 Combination of cost of resistance and differential mortality in malaria-infected mosquitoes – evolution proofing

**Figure 30. Fitness of resistant mosquitoes relative to susceptibles for a late-life acting insecticide for various costs of resistance and differential efficacy against malaria-infected mosquitoes.**

When relative fitness >1, resistance spreads, when relative fitness is <1, resistance will not spread, even when present in a population (complete evolution-proofing). Plotted values are for an ADI insecticide which kills mosquitoes on contact during or after their 4<sup>th</sup> gonotrophic cycle, reducing infectious bites by 95% in susceptible populations. Differential mortality is the proportionate mortality produced by the ADI in malaria uninfected mosquitoes compared to that in malaria-infected mosquitoes. Costs of resistance accrue as additional daily mortality rates.



The results summarised in Figure 30 are the fitness values for resistant mosquitoes relative



to susceptibles in a population subject to a 4-cycle ADI with pre-bite delivery at 80% coverage. The results assume a range of values for differential mortality in malaria-infected mosquitoes, combined with various costs of resistance. It can be seen that very low COR can result in relative fitness values for resistant mosquitoes below 1.00, meaning that resistant mosquitoes are less fit than susceptibles and resistance cannot spread. When COR is combined with differential mortality in malaria-infected and uninfected mosquitoes, a COR as low as 0.43% can give evolution proofing.

### 3.6 Sensitivity Analysis

To explore the significance of the parameters used in our evaluation we conducted a sensitivity analysis. In most cases this gave results differing quantitatively from our base case, but in all cases results are qualitatively consistent with our conclusions.

#### 3.6.1 Proportion non-human feeds

In a given gonotrophic cycle, a mosquito which feeds on a non-human host cannot catch malaria nor give an infectious bite to a human. We also assume that insecticides are delivered using methods which mean mosquitoes are only exposed to them when attacking human hosts.

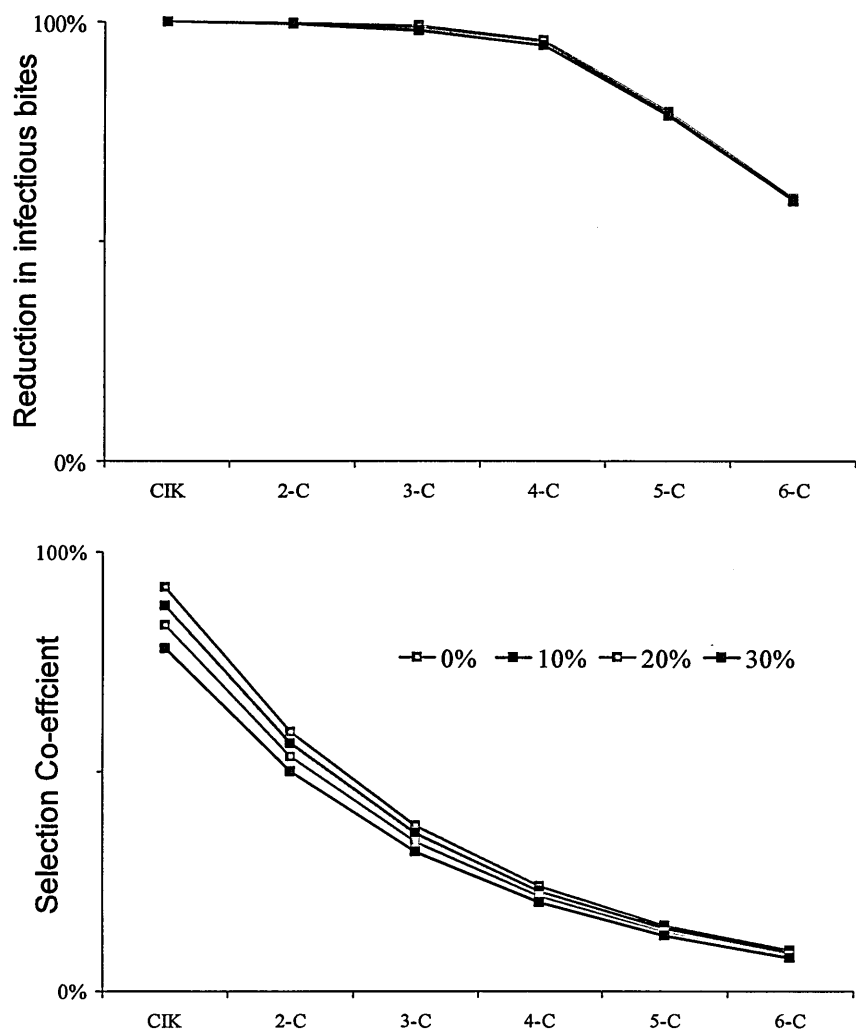
**Table 14 Summary of LRS and AIB values without interventions, relative to base case values for various probabilities of feeding on a non-human host**

<i>Results for untreated populations</i>	<i>relative to base case values</i>	
	AIB	LRS
0% probability feeds on non human host	1.44	1.00
10% probability feeds on non human host	1.17	1.00
20% probability feeds on non human host	0.93	1.00
30% probability feeds on non human host	0.72	1.00

As can be seen from Table 14, the probability of feeding on a non-human host can make a substantial difference to EIR. For example, a species feeding entirely on humans would generate 44% more infectious bites per mosquito per unit of time than a species which has a 17% probability of feeding on a non-human host, our base case assumption. Without

intervention, there is no effect on fitness. However, since we assume that mosquitoes are only exposed to insecticides when choosing to feed on a human host, the probability of feeding on a non-human host does affect fitness values when considering the effects of interventions.

**Figure 31 Non-human feeding propensity**  
Reduction in EIR and selection coefficient for CIKI and range of ADIs, assuming 0%, 10%, 20% or 30% probability of choosing a non-human host for any given feeding attempt.

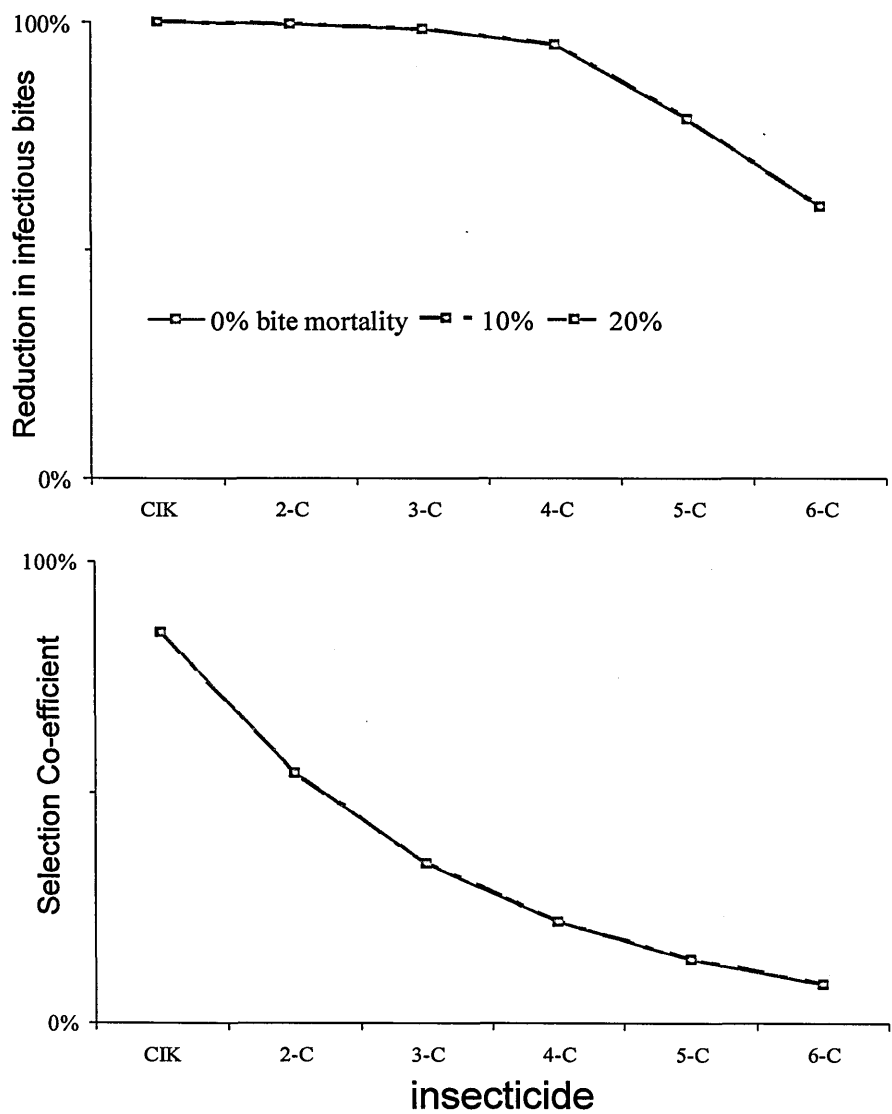


Although non-human feeding propensity has no material effect on comparative reductions in EIR (Figure 31, top panel), it can be seen (Figure 31, bottom panel), that it does affect resistance management, higher non-human feeding propensity giving lower selection coefficients and hence better resistance management. This effect is more significant for CIKI and low effective age ADIs.

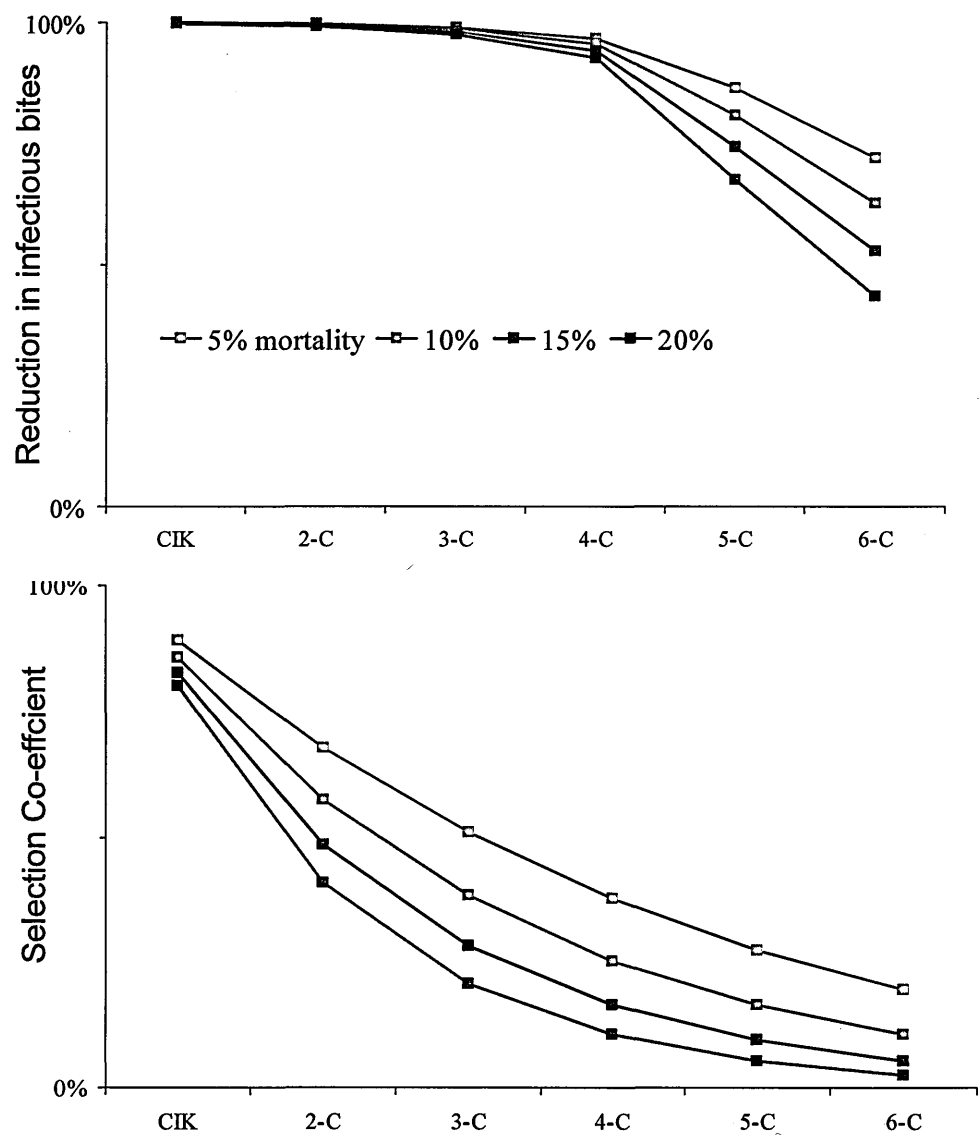
### 3.6.2 Natural mortality rates

We assume that mosquitoes are subject to a daily background mortality rate, plus additional mortality related to feeding and/or egg laying of 10% per attempted bite, half pre-bite, and half post bite. It can be seen from Figure 32 that, for the present analysis, the division of mortality per cycle between the daily background mortality rate and the feeding-related figure has no effect on our results when the per-cycle mortality is maintained at a constant level. From Figure 33 and Figure 34 however, it is clear that changing either mortality assumption independently, so that the overall mortality per cycle changes , does affect our results for both EIR reduction and resistance management.

**Figure 32 Constant per cycle mortality with varying mixture of background and bite-related mortality**



**Figure 33 Background mortality rate sensitivity**  
 Reduction in EIR and selection coefficient for CIKI and range of ADIs, assuming 5%, 10%, 15% or 20% daily background mortality rates

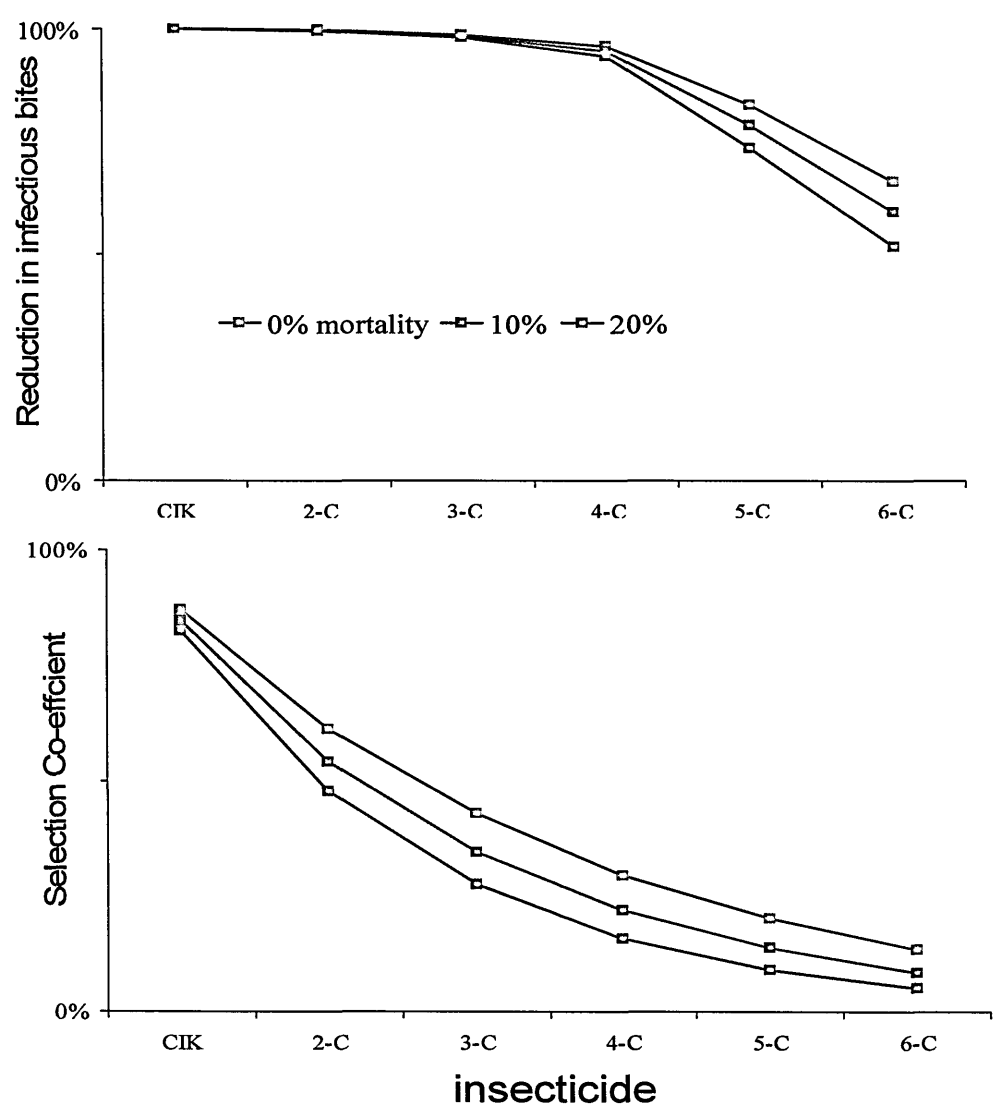


**Table 15 Summary of LRS and AIB values without interventions, relative to base case values for various natural mortality assumptions**

<i>Results for untreated populations</i>	<i>relative to base case values</i>	
	EIR	LRS
Constant per cycle mortality with 0% bite mortality	1.01	1.00
Constant per cycle mortality with 20% bite mortality	0.99	1.00
5% per day background mortality rate	3.33	1.83
10% per day background mortality rate	1.35	1.15
15% per day background mortality rate	0.59	0.79
20% per day background mortality rate	0.27	0.58
0% feeding mortality rate	1.88	1.35
20% feeding mortality rate	0.53	0.76

EIR and LRS are both sensitive to daily background mortality and blood-feeding related mortality rates (Table 15). Higher values for either mortality rate result in a reduction in RAIB, and a decrease in the selection co-efficient (Figure 33 and Figure 34). The loss of EIR reduction is minimal for CIKIs and ADIs with effective ages in the most useful range, increasing markedly with higher ADI effective ages. There is a reduction in selection coefficients with higher mortality for all evaluated insecticides, with a proportionately larger effect for later effective age ADIs.

**Figure 34 Blood-feeding mortality sensitivity**  
Reduction in EIR and selection coefficient for CIKI and range of ADIs, assuming 0%, 10% or 20% bite-related mortality rates.



If a constant per cycle mortality is maintained, it can be seen that varying the proportion of mortality contributed from blood-feeding versus daily background mortality rates has little

effect on EIR or LRS (Table 15) and no impact on reduction in EIR nor selection coefficient (Figure 32). It is thus the overall mortality per cycle, determined by daily background mortality, feeding-related mortality and days per cycle, rather than the allocation of mortality between daily and feeding-associated mortality which affects our key outcomes. The optimum combinations of transmission control and resistance management would seem to be achievable in contexts with high natural mortality per feeding cycle, which offer improved selection coefficients at little or no cost in terms of EIR reduction, for ADIs with effective ages in the most useful range.

### **3.6.3 Mosquito senescence**

We have followed the example of many previous analysts [51,61,62] in using a simple assumption of constant natural background mortalities. Increasingly, however, arguments are being made for the importance of understanding mosquito life-tables and reflecting them in vector-control models [63-65]. Since our study specifically considers differential induced mortality in different mosquito age-classes, it seems appropriate to consider whether age-linked changes in background mortality rates might affect our results.

For this analysis, however, we cannot simply use mortality rates generated from laboratory data. Mortality in laboratory mosquitoes is generally much lower than that observed in the field [51,63] and the resistance management benefit of the LLA principle is predicated on the high mortalities experienced by the target populations of wild vectors. Figure 33 and Figure 34, for example, illustrate the importance of natural mortality for the selection coefficients achievable with ADIs. Although a number of mark and recapture experiments are in the literature, there is not yet any clear consensus regarding the range of age-linked variation in mortalities experienced by wild mosquitoes. We therefore chose to use a model generated by Styer et al [64], based on large scale laboratory studies, and providing a fully-specified model, adjusting the values of some parameters to reflect field rather than laboratory overall mortalities.

Using the logistic model fitted by Styer et al for mortality rates in female mosquitoes, we changed the model parameters to give per cycle mortality across the population matching our base case value, by adjusting either the initial age-linked mortality rate (parameter *a* in the Styer model), the incremental age-independent mortality rate (parameter ‘*c*’ in the Styer model) or both the initial age-linked mortality and the exponential rate of increase in the mortality rate (parameter ‘*b*’ in the Styer model). The mortality rates generated by adjusting each parameter are plotted in panel A of Figure 35. Using parameter ‘*c*’ maintains the curve produced by the original model, but displaces it upwards, as would be expected. Increasing the initial age-dependent mortality rate, parameter ‘*a*’, to achieve the same overall mortality produces a different mortality pattern. Adjusting the initial mortality rate by half the amount needed to achieve our required per-cycle mortality, and then achieving the required total mortality by adjusting the exponential rate of change, parameter ‘*b*’ gives a much steeper s-shaped survival curve, and can probably be considered an extreme case. It can be seen that, given the relatively low numbers of older mosquitoes, later high mortality rates can only offset a relatively small early-life reduction in mortality versus a constant age-independent rate, if the same overall mortality rate per cycle is to be achieved.

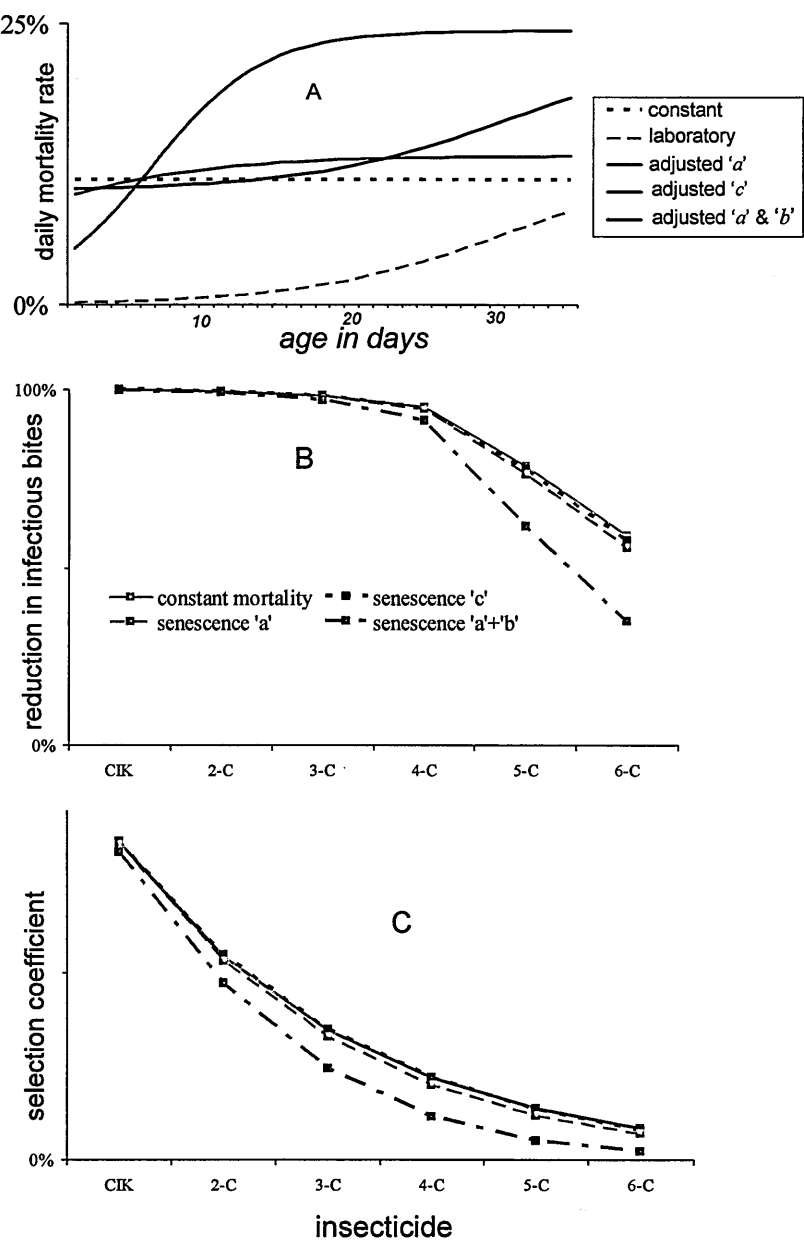
These cases represent various possible ways that the senescence values observed in the laboratory might inform age-linked mortality in the field. Age-independent mortality could include mortality from external sources such as predation, starvation, dehydration, contact with sticky surfaces etc. The general stresses of life in a fluctuating heterogeneous environment might increase the level of inherent mortality and/or the rate at which it increases with age.

**Table 16 Summary of LRS and AIB values without interventions, relative to base case values for various mosquito senescence assumptions**

<i>Results for untreated populations</i>	<i>relative to base case values</i>	
	EIR	LRS
senescence using adjusted parameter ‘ <i>c</i> ’	1.03	1.04
senescence using adjusted parameter ‘ <i>a</i> ’	0.90	1.00
senescence using adjusted parameter ‘ <i>a</i> + <i>b</i> ’	0.50	1.01

It can be seen from Table 16 that the senescence mortality profiles considered, although all resulting in the same average mortality per cycle across the whole population, nonetheless produced variation of more than 50% in the calculated EIRs for untreated populations.

**Figure 35 : Mosquito senescence scenarios**  
 Plots in panel A show the daily mortality rates assumed for constant mortality, laboratory mortality, and three senescence scenarios based on the logistic model fitted by Styer et al to laboratory mortality rates [64], and using mortalities calculated by adjusting either parameter ‘a’, initial age-dependent mortality rate, parameter ‘c’, age-independent mortality rate, or parameter ‘b’, the exponential rate of increase for age dependent mortality, to give the same overall mortality per cycle (35%) as our constant base case mortality assumption, in an untreated susceptible population. Reduction in EIR (panel B) and selection coefficients (panel C) for CIKI and ADIs with effective ages of 2, 3, 4, 5 and 6 cycles using constant mortality and three senescence scenarios.





It can be seen from Figure 35 (panels B and C) however, that because we use comparative values for key metrics, the EIR reductions and selection coefficient results for all but the most extreme of the senescence profiles tested are not materially different to those calculated with a constant mortality rate. For the more extreme case, EIR reductions are lower with higher ADI effective ages, but little affected for the more useful ADIs, whilst selection coefficients are improved compared to those calculated using a constant mortality rate. In assessing the importance of age-linked mortality effects, they clearly have a potentially large impact on EIR, but there is perhaps a balance to be achieved between ignoring them inappropriately, and over-emphasising them whilst ignoring the more significant differences between mortality rates in the field and the laboratory, which in turn serve to reduce the impact of detailed mortality profiles with respect to older mosquitoes.

**3.6.4 Probability feed on human host results in *Plasmodium* infection of mosquito**

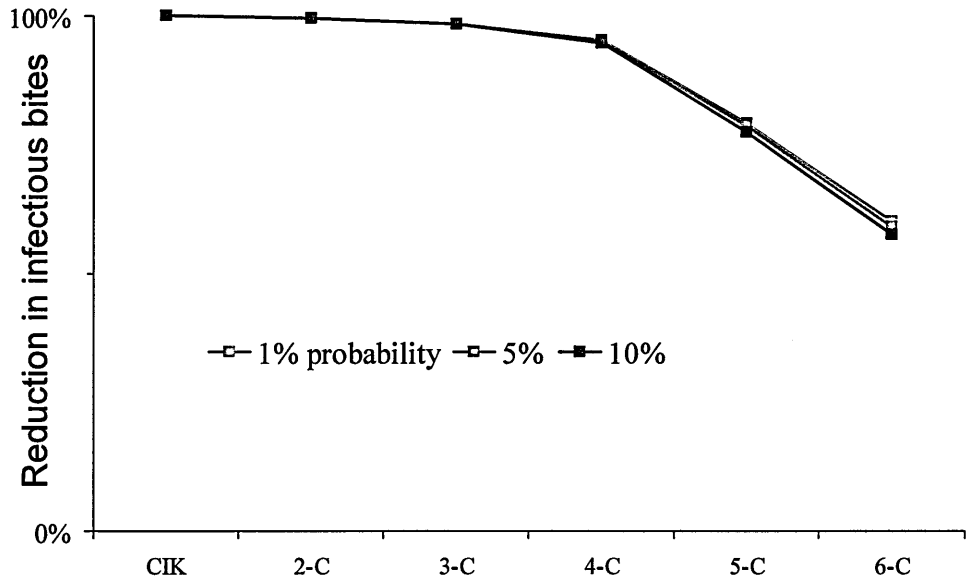
It can be seen from Table 17 that the assumed probability of a mosquito acquiring a *Plasmodium* infection when biting a human host has a substantial impact on EIR. From Figure 36, however, it can be seen that the relative reductions in EIR achievable with a given intervention are essentially unaffected by this parameter.

**Table 17 Summary of LRS and AIB values without interventions, relative to base case values for various probabilities of a mosquito acquiring a *Plasmodium* infection when feeding on a human host.**

<i>Results for untreated populations</i>	<i>relative to base case values</i>	
	EIR	LRS
1% probability of catching malaria when feeding on human host	0.24	1.00
5% probability of catching malaria when feeding on human host	1.16	1.00
10% probability of catching malaria when feeding on human host	2.21	1.00

**Figure 36 Malaria prevalence sensitivity**

Reduction in EIR and selection coefficient for CIKI and range of ADIs, assuming 1%, 5%, or 10% probability of mosquito acquiring a *Plasmodium* infection when feeding on a human host.



**3.6.5 Mosquito mortality and fecundity effects of *Plasmodium* infection**

Although there is still debate around this issue [66], it is increasingly clear that *Plasmodium* has pronounced effects on mosquito fitness, by affecting both survival and fecundity [67,68]. We here consider the extent to which such effects might alter our conclusions with respect to LLA insecticides.

**Table 18 Summary of LRS and AIB values without interventions, relative to base case values with various assumptions for the effects of *Plasmodium* infection on mosquito mortality and fecundity**

Results for untreated populations	relative to base case values	
	EIR	LRS
37.5% increase in bite mort for malaria infectious mosquitoes	0.93	1.00
37.5% increase in 20% bite mort for malaria infectious mosquitoes	0.47	0.76
reduced mortality and fecundity in infected mosquitoes	1.98	1.00
reduced mortality and fecundity +35% bite-related mort in infected mosquitoes	1.82	0.99
3 x base mortality in oocyst stage infection	0.28	0.96

Anderson et al [67] found an increase in feeding associated mortality of 37.5% in sporozoite positive (infectious) mosquitoes in the field. Using this estimate in

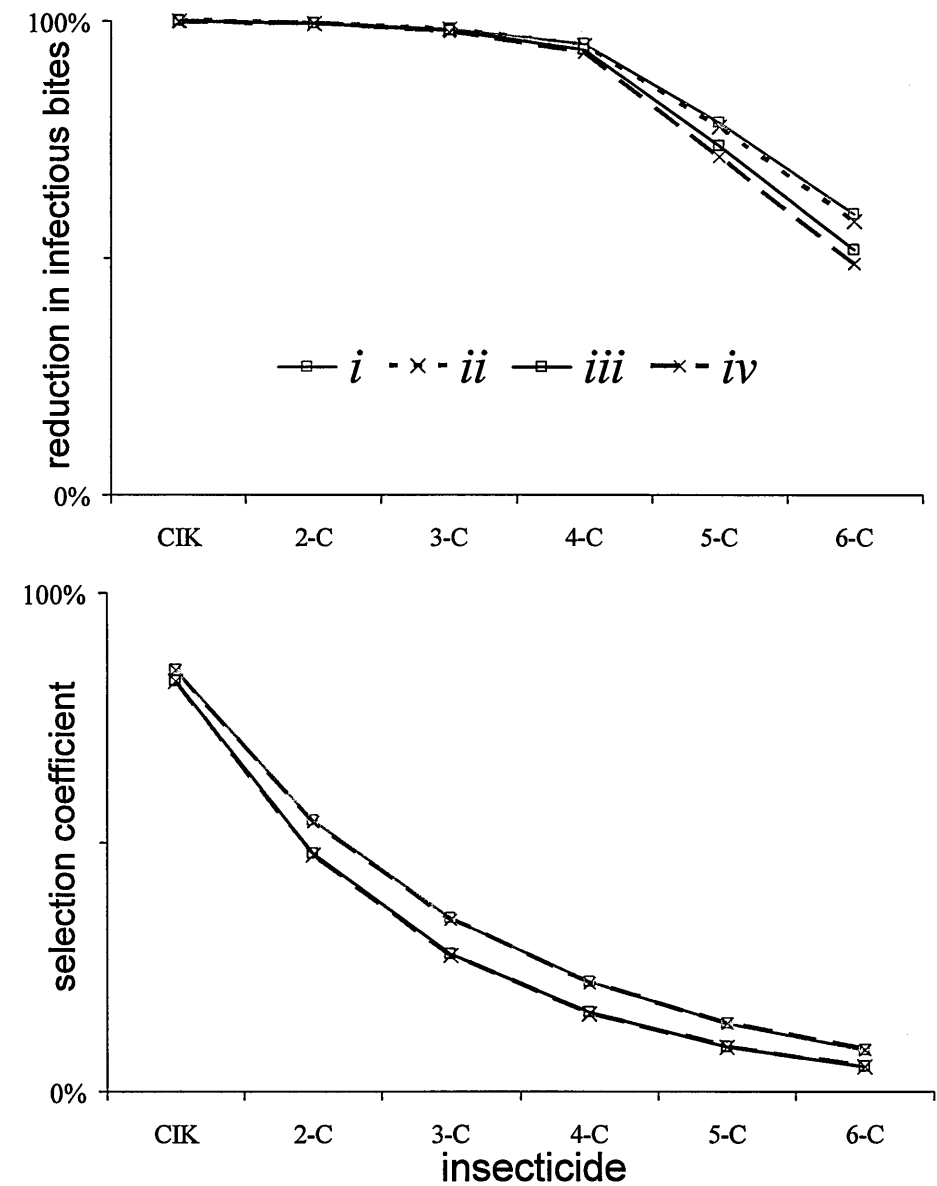
combination with our base 10% bite-related mortality assumption we did not find any material change in our calculated values for EIR reduction or selection coefficient (Figure 37, plots *i* and *ii*). As a proportionate adjustment, this effect would be expected to be more significant in combination with higher bite-related mortalities; we therefore also considered a 37.5% increase in bite-related mortality for malaria infectious mosquitoes using baseline bite-related mortality of 20%. This reduced the EIR relative to our base case by 53% (Table 18) compared to a reduction of 47% for a 20% feeding-related mortality case without incremental malaria-associated mortality (Table 15), whilst showing little effect on EIR reduction and selection coefficient values (Figure 37 plots *iii* and *iv*).

Vézilier et al [68], working with a natural mosquito-malaria system, found enhanced survival linked with reduced egg-production in *Plasmodium* infected mosquitoes. Taking the survival figure for the mosquito strain showing the strongest survival effect (55% reduction in background daily mortality), and the average fecundity reduction (30%), we find that, whilst EIR under these assumptions is approximately double that for our base case (Table 18), they produce a slight improvement in the transmission reduction potential of later effective age ADIs, without increasing the selection coefficient for a given insecticide (Figure 38).

Much work seeking to establish the effects of *Plasmodium* infection on mosquitoes has been done using *Plasmodium*/mosquito combinations not found in the wild, under idealised laboratory conditions. Aboagye-Antwi et al [69] used wild-caught blood fed *Anopheles gambiae* s.s. subject to restricted water access to explore the effects of natural infections with *Plasmodium falciparum* on females experiencing stress associated with sub-optimal conditions. They found that females subject to hydric stress and carrying *Plasmodium* oocysts showed substantially higher mortality than those uninfected or carrying sporozoites. Based on their results we consider a three-fold increase in daily background mortality for infected oocyst-stage females and find that this assumption gives an EIR approximately 70% below our base case value (Table 18). The proportionate reductions in

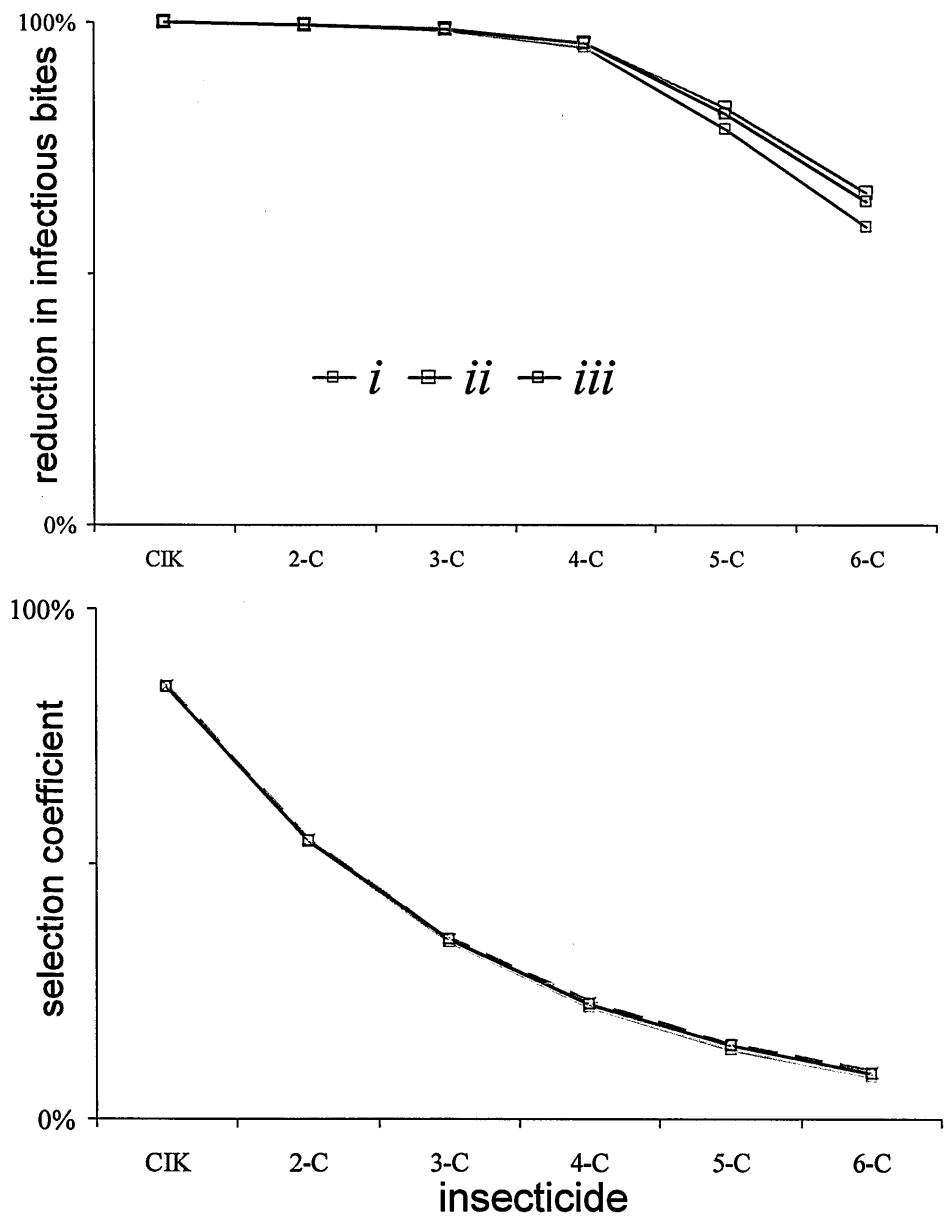
EIR with conventional and ADI insecticides are completely unchanged by this assumption, however, and selection coefficients show only a very slight improvement (Figure 39).

**Figure 37** Effect of differential bite-related mortality in malaria infectious mosquitoes  
 Plotted values are the proportionate reduction in EIR (top panel) and selection coefficient (bottom panel) for conventional and age-dependent instant-kill insecticides at 80% coverage with pre-bite delivery. Plots are for 10% (*i*) and 20% (*ii*) bite-related mortality for all mosquitoes, and 10% (*iii*) and 20% (*iv*) bite-related mortality assuming 37.5% higher bite-related mortality in mosquitoes with an infectious malaria infection.



**Figure 38 Effect of reduced mortality and fecundity in *Plasmodium* infected mosquitoes**

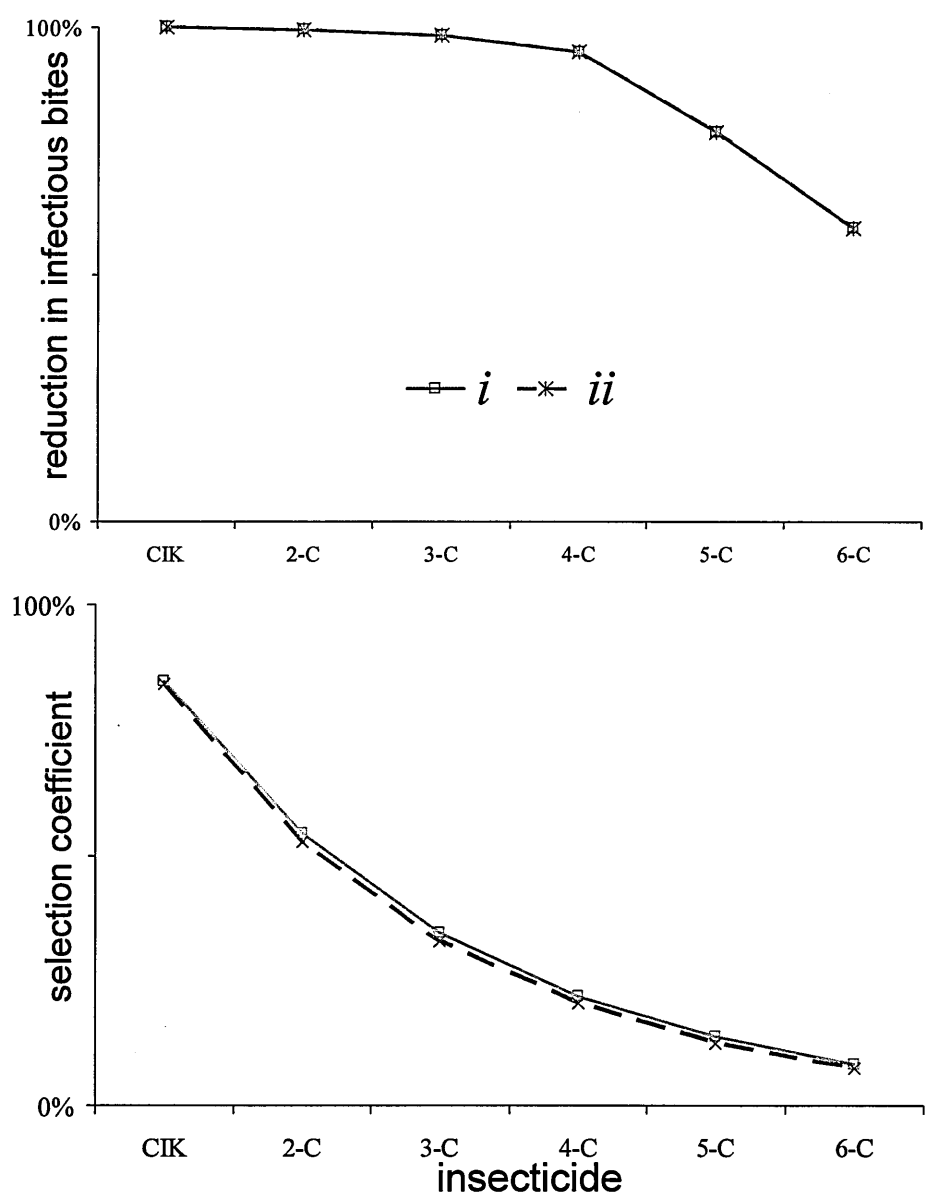
Plotted values are the proportionate reduction in EIR (top panel) and selection coefficient (bottom panel) for conventional and age-dependent instant-kill insecticides at 80% coverage with pre-bite delivery. Plots are for (i) base case and (ii) 55% reduction in background mortality with 30% reduction in fecundity for *Plasmodium* infected mosquitoes and (iii) 55% reduction in background mortality with 30% reduction in fecundity for *Plasmodium* infected mosquitoes plus 35% additional bite-related mortality for all malaria infectious mosquitoes.



Our conclusions seem to be robust to a range of assumptions about the effect of *Plasmodium* infection in mosquitoes, although such effects can have a substantial impact on the calculated EIR values, and may be very important to many aspects of malaria epidemiology, the efficacy of some other types of control measure, and to calculated thresholds for eradication strategies.

**Figure 39 Effect of increased mortality in pre-infectious *Plasmodium* infected mosquitoes**

Plotted values are the proportionate reduction in EIR (top panel) and selection coefficient (bottom panel) for conventional and age-dependent instant-kill insecticides at 80% coverage with pre-bite delivery. Plots are for (i) base case and (ii) assumed 3-fold increase in background mortality for pre-infectious malaria infected mosquitoes.



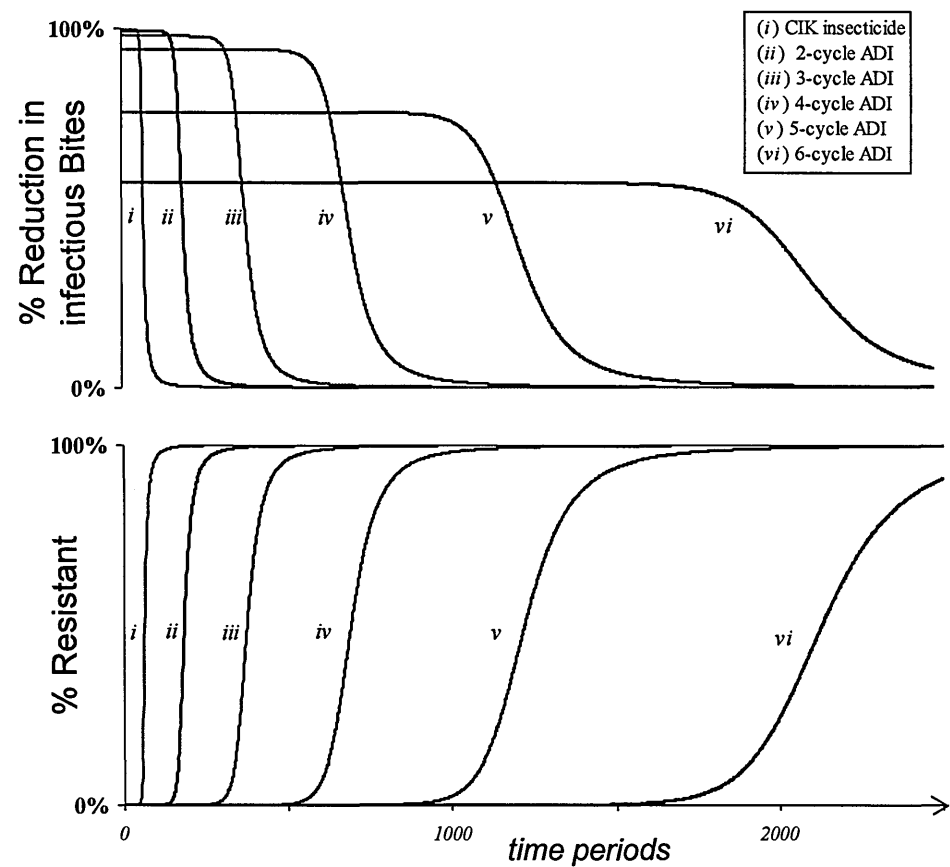
### 3.6.6 Constant population size *versus* constant recruitment

Our analysis assumes that the rate at which new adults join the population remains constant with and without insecticide use, and through all modelled time periods. This would be consistent with complete density dependence at the juvenile stage, so that reduced egg production from a population treated with a CIKI or ADI would not result in a material change in the numbers of emerging adults. From a modelling perspective this gives a

population size which varies with post-emergence adult mortality only.

To test the sensitivity of our results to this assumption we ran a version of the PM model amended to maintain a constant adult population size.

**Figure 40 Results from the PM showing the spread of resistance over time, and the consequent loss of reduction in EIR assuming constant adult population size.** Resistant phenotypes as a proportion of the population (bottom panel) and consequent loss of reduction in population level infectious bites (top panel) for a CIKI and ADIs with 2, 3, 4, 5 and 6 cycle effective age, assuming constant population size at the start of all time periods.



Comparison between the base case results in Figure 18, with constant adult recruitment, and the results in Figure 40 confirms that our assumption of constant rate of recruitment to the adult population is not key to our conclusions.

### 3.6.7 Dominant/recessive resistance alleles

See Chapter 6 for additional discussion of this issue.

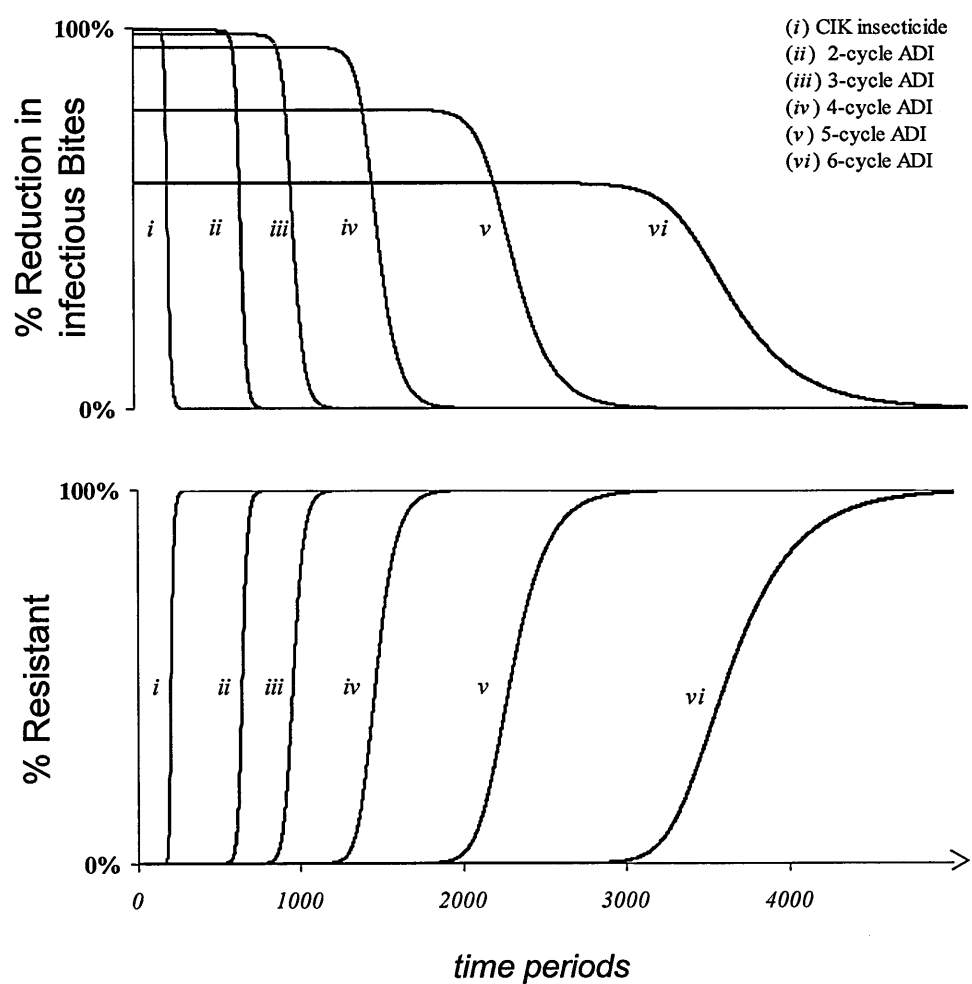
#### 3.6.7.1 Recessive resistance

Our analysis assumes that, for all insecticides, resistance is controlled by a single, dominant allele. Both dominant and recessive modes of action have been identified for

resistance to existing insecticides [70,71], so we here examine whether the assumption of dominance is key to our conclusions.

It can be seen from Figure 41 that, assuming resistance is controlled by a recessive allele for all insecticides predictably gives slower rates of spread of resistance, but does not change our fundamental conclusions regarding the relationship between transmission control and resistance management.

**Figure 41 Results from the PM showing the spread of resistance over time, and the consequent loss of reduction in EIR assuming resistance allele is recessive.** Resistant phenotypes as a proportion of the population (bottom panel) and consequent loss of reduction in population level infectious bites (top panel) assuming recessive resistance allele, for a conventional instant-kill insecticide and ADIs with effective ages of 2, 3, 4, 5 and 6 cycles.

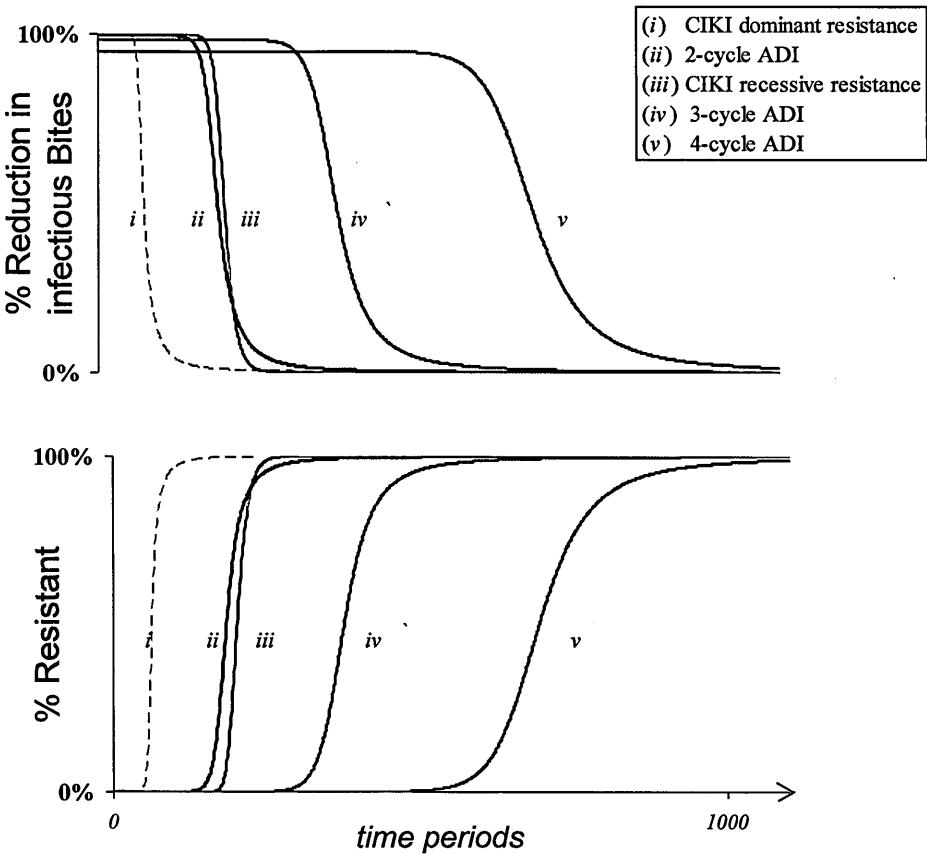




3.6.7.2 Recessive resistance for conventional insecticides, dominant for LLAs

In the absence of knowledge regarding resistance mechanisms for theoretical LLA insecticides, we have compared the spread of resistance assuming equivalent mechanisms for conventional and LLA insecticides. Clearly the situation which would minimise relative resistance management benefits for LLAs would be dominant resistance for the LLA, with recessive resistance for conventional instant-kill insecticides. Making this comparison (Figure 42) shows that all but the earliest-acting LLAs would continue to offer resistance management benefits compared to CIKIs.

**Figure 42 Comparison of speed of spread of resistance over time with dominant LLA resistance allele and recessive CIKI resistance allele**  
Resistant phenotypes as a proportion of the population (bottom panel) and consequent loss of reduction in population level infectious bites (top panel) assuming dominant resistance allele for ADIs with effective ages of 2, 3, and 4 cycles, and dominant or recessive resistance allele for conventional instant-kill insecticide.

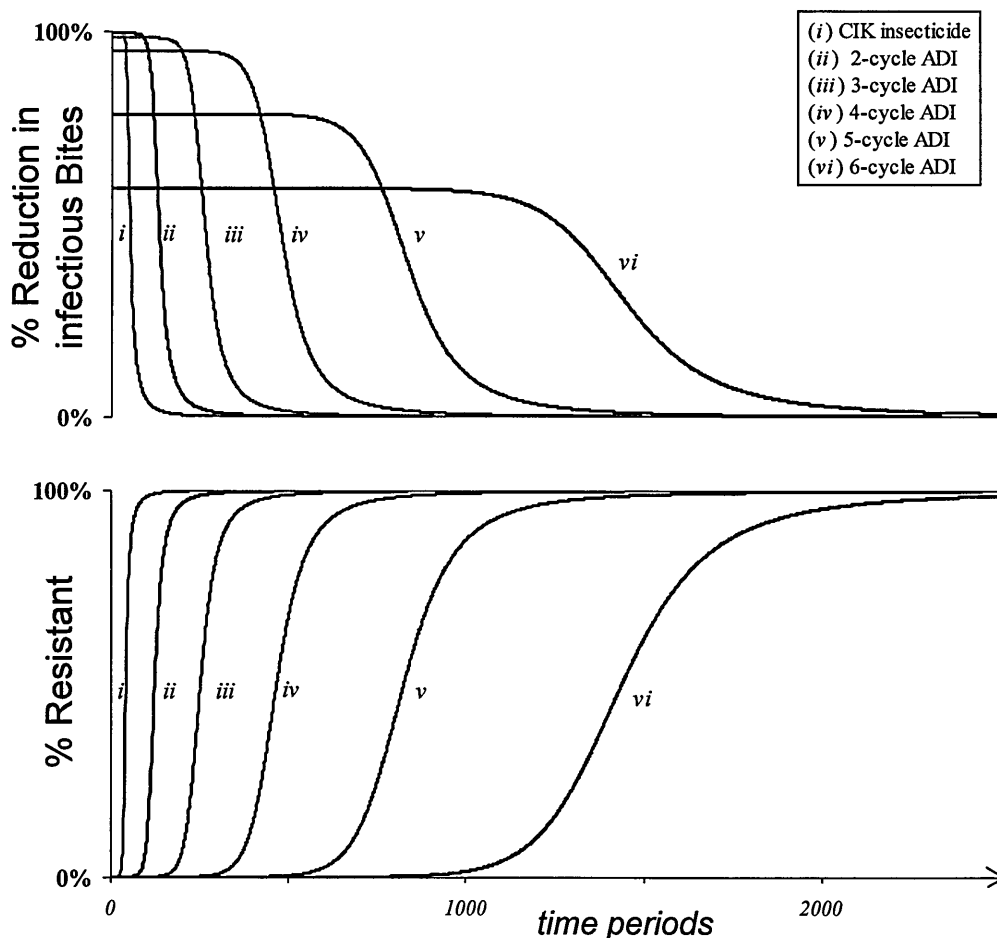


### 3.6.8 Assumed starting level of resistants in the population

It is not possible to predict how long resistance will take to arise in a population, and all our results compare the speed of spread once resistance alleles are present. Our base case assumption is that resistance alleles are initially present as rare heterozygotes, comprising one per billion of the mosquito population. We here assess whether the start point materially affects the comparative rates of spread for CIKIs and ADIs, given that the analysis is primarily interested in the process of resistance spreading from an initially low level in the population.

**Figure 43 Comparison of speed of spread of resistance for CIKI and ADIs assuming  $10^{-6}$  initial prevalence of heterozygotes in population**

Resistant phenotypes as a proportion of the population (bottom panel) and consequent loss of reduction in population level infectious bites (top panel) assuming initial population has  $10^{-6}$  heterozygotes. Plots are for CIKI and ADIs with effective ages of 2, 3, 4, 5 and 6 cycles.

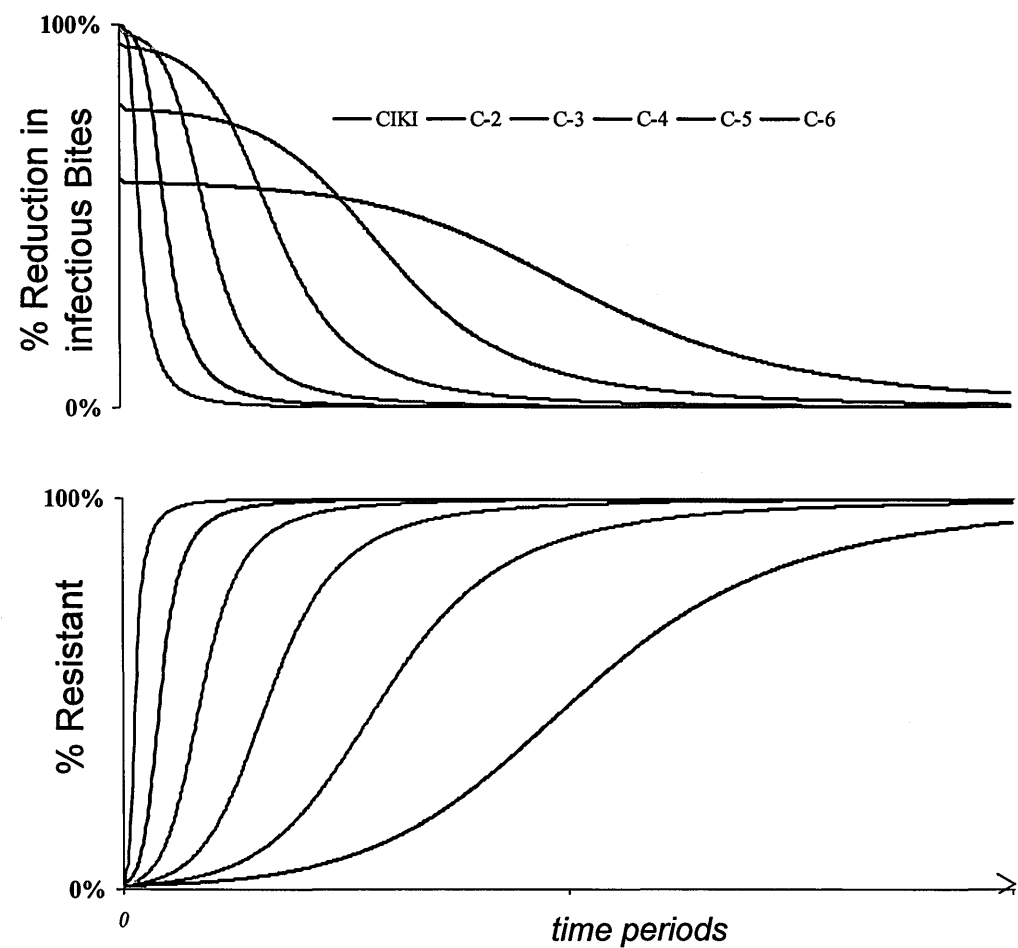


Comparison between Figure 18 and Figure 43 shows that a 1,000-fold increase in the

assumed initial level of heterozygotes in the population, to 1 per million, does not materially change the relative useful lives of a CIKI and ADIs with effective ages of 2, 3, 4, 5 and 6, before the spread of resistance eliminates transmission control. Absolute times between the start of the evaluation and any given proportion of resistants in the population are predictably shorter for all insecticides.

**Figure 44 Comparison of speed of spread of resistance for CIKI and ADIs assuming 1% initial prevalence of heterozygotes in population**

Resistant phenotypes as a proportion of the population (bottom panel) and consequent loss of reduction in population level infectious bites (top panel) assuming initial population has 1% heterozygotes. Plots are for CIKI and ADIs with effective ages of 2, 3, 4, 5 and 6 cycles.

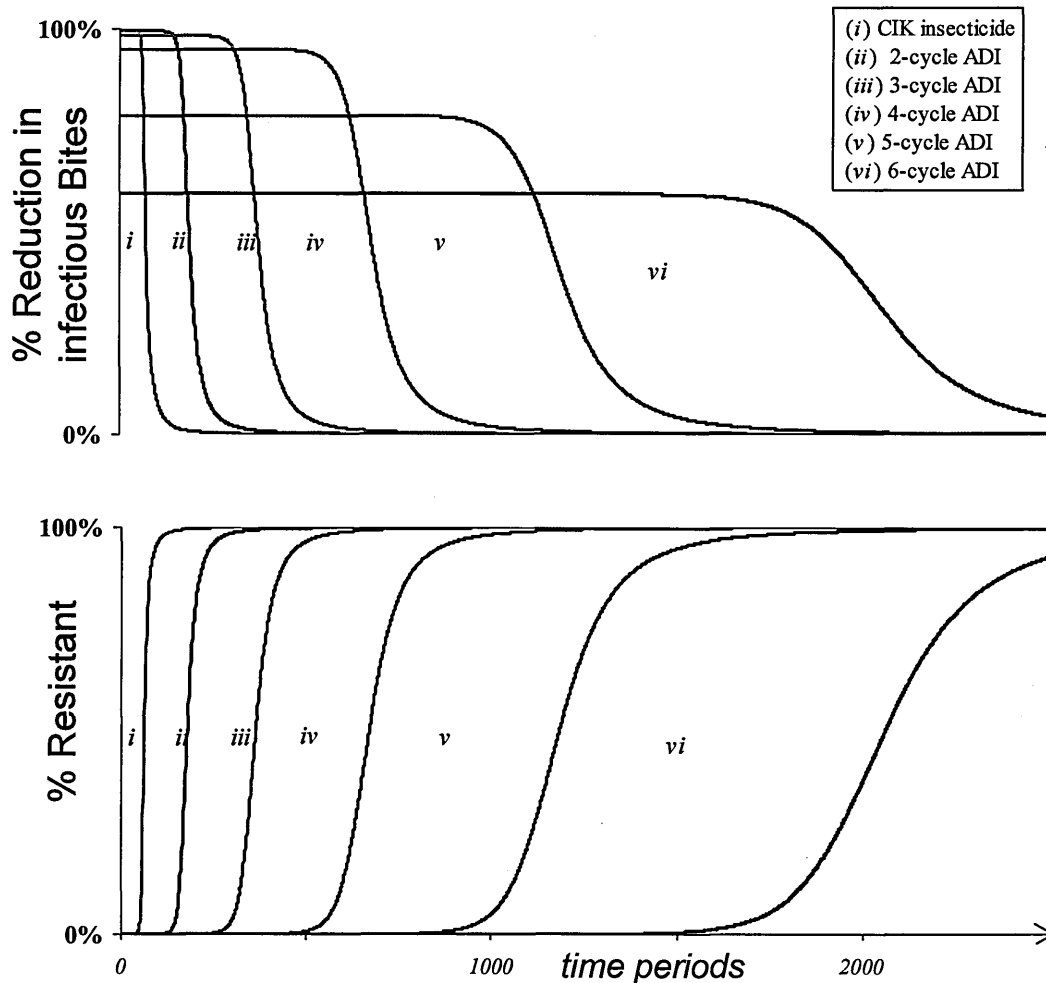


When the proportion of heterozygotes is increased to 1%, the profile of the spread of resistance changes (Figure 44), but, again, the relative times to loss of effectiveness, defined as any given level of resistance in the population, still show a relationship between the CIKI and ADIs consistent with our conclusions.

We have assumed that rare alleles will primarily be present in heterozygotes. However, it can be seen from Figure 45 that changing this assumption and assuming instead that the resistants initially present in the population are all homozygotes, does not affect our conclusions.

**Figure 45 Comparison of speed of spread of resistance for CIKI and ADIs assuming  $10^{-9}$  initial prevalence of homozygotes in the population**

Resistant phenotypes as a proportion of the population (bottom panel) and consequent loss of reduction in population level infectious bites (top panel) assuming initial population has  $10^{-9}$  heterozygotes. Plots are for CIKI and ADIs with effective ages of 2, 3, 4, 5 and 6 cycles

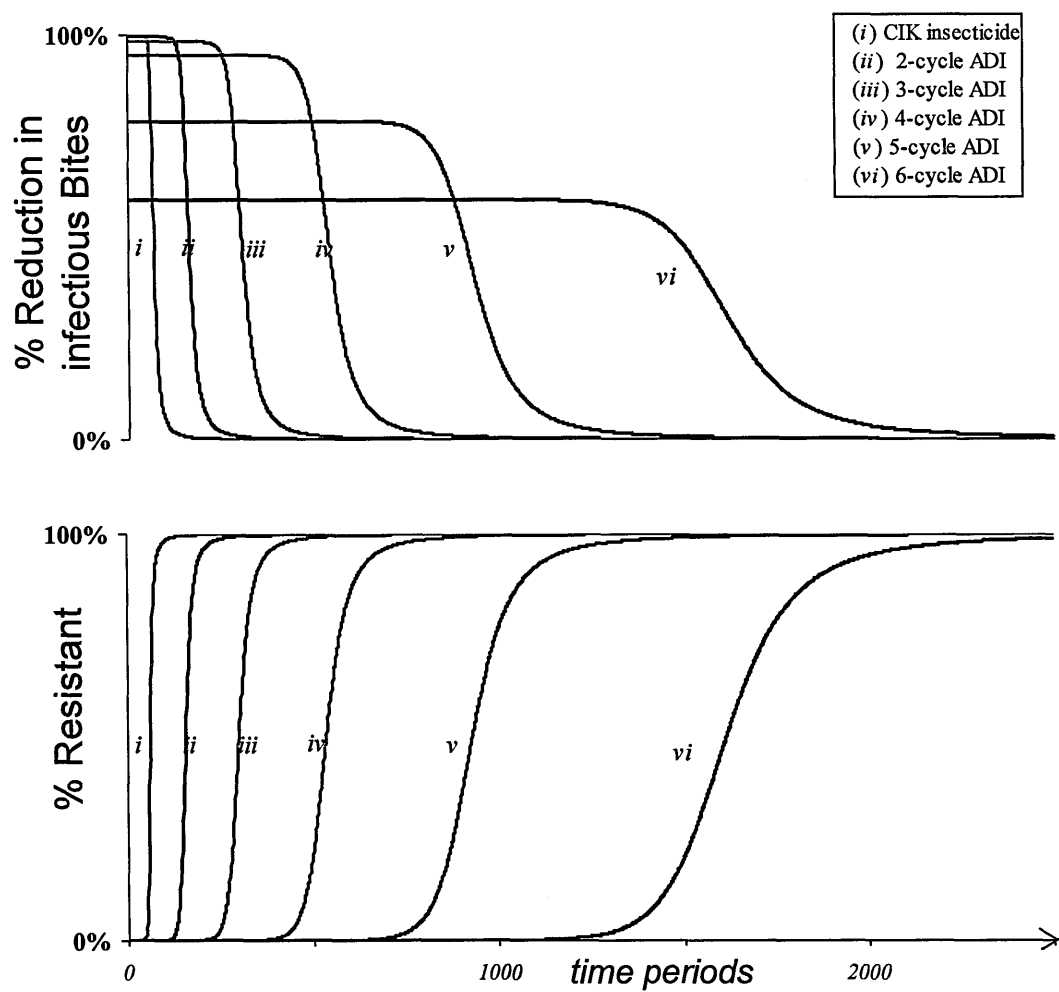


### 3.6.9 Male population matches genotype of new adults or of females

We have assumed that females mate once, shortly after emerging as adults, and that they primarily mate with males emerging at around the same time. The validity of this assumption depends on males having a short reproductive life. If instead males survive and continue to mate with new females over an extended period, the allele frequencies among

mating males will be different to that which we have assumed. To explore the significance of this, we adjusted the model and repeated our analysis assuming that, at the time of mating, the male population has the same allele frequencies as the total adult female population, rather than that of newly-emerged adults only.

**Figure 46 Comparison of speed of spread of resistance for CIKI and ADIs assuming males have same allele frequencies as adult female population**  
Resistant phenotypes as a proportion of the population (bottom panel) and consequent loss of reduction in population level infectious bites (top panel) assuming that mating males have the same genetic mix as the adult female population. Plots are for CIKI and ADIs with effective ages of 2, 3, 4, 5 and 6 cycles



It can be seen from comparison between Figure 46 and Figure 18 that resistance spreads faster with male genotype proportions matching those of adult females rather those of newly emerged adults, but, again, the relative times to spread of resistance remain consistent with our conclusions.

### 3.6.10 Geographic data sets

We have used a data set based on averages of field data from four geographic locations, which vary by climate, vector species etc. Here we assess whether the average values provide a reasonable basis for our evaluation, or whether the geographic data sets considered individually produce results inconsistent with those generated using the average values.

**Table 19 Geographic data sets**

	Kankiya	Kaduna	Namawala	Butelgut	Average
Probability of acquiring malaria infection when biting a human host	2%	6%	2%	7%	4%
Length of feeding cycle / days	3.00	2.00	2.70	3.70	2.85
Instantaneous daily mortality rate	6%	10%	17%	14%	12%
Per feeding cycle mortality	17%	19%	40%	43%	28%
Probability attacks non-human host	0.25	0.10	0.05	0.28	0.17
Number of cycles from malaria infection to infectious bite	3	5	4	2	3

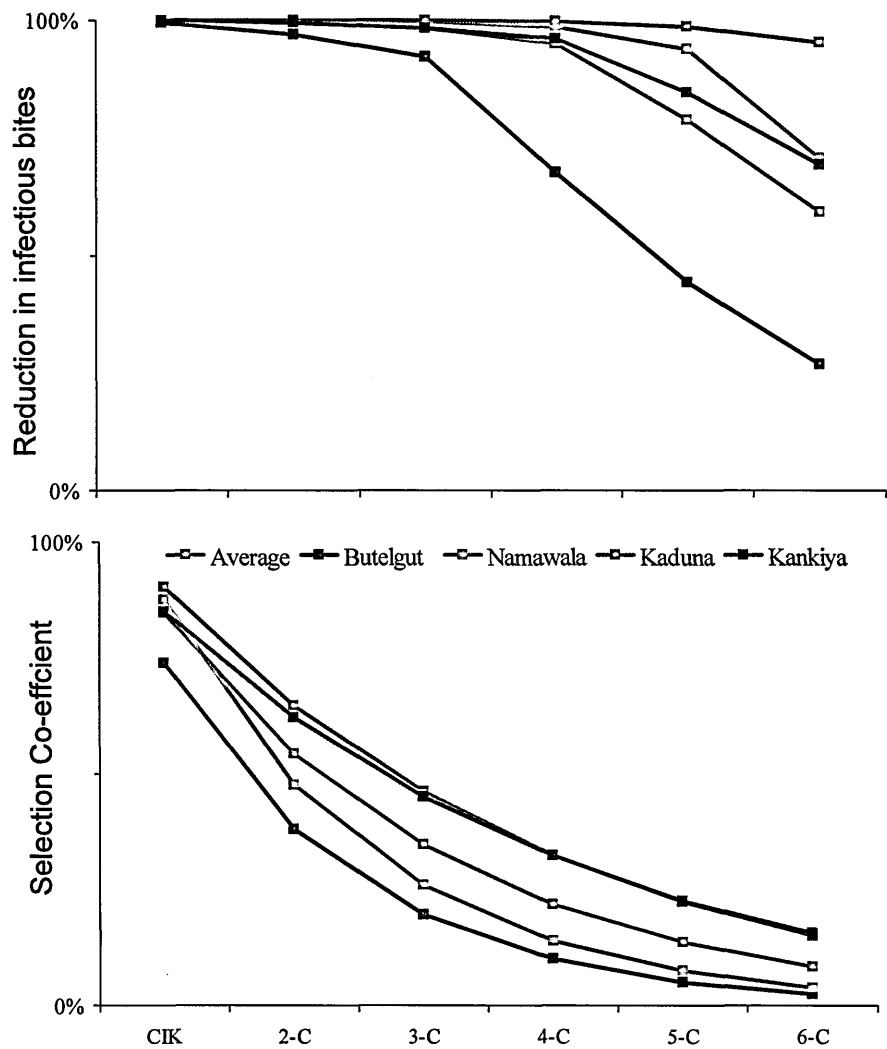
**Table 20 Summary of LRS and AIB values without interventions, relative to base case values, using individual geographic data sets**

<i>Results for untreated populations</i>	<i>relative to base case values</i>	
	AIB	LRS
Kankiya	1.18	1.57
Kaduna	1.30	1.44
Namawala	0.11	0.67
Butelgut	0.73	0.59

It can be seen from Table 20 that AIB and LRS results vary widely between the four geographic data sets. The absolute values of AIB and LRS do not, however give an indication of the relative reductions in EIR and selection coefficients associated with insecticide use for a given data set, as can be seen in Figure 47, where the two locations offering the highest EIR reductions are Namawala and Kaduna, which have respectively, the lowest and highest absolute values for AIB.

The reductions in infectious bites across the different ADI effective ages (Figure 47, top panel), are consistent with the results in Figure 24 for all four locations, indicating that optimum disease control and resistance management combinations are offered at  $\pm 1$  cycle compared to the malaria development period. A steep loss of EIR reduction occurs for ADIs with effective age above 4 for Kankiya, which has a 3 cycle malaria development period, and above five for Namawala which has a four-cycle malaria development period. For Kaduna, reduction in EIR is well-maintained for ADIs with effective ages up to 6, consistent with its malaria development period of 5 gonotrophic cycles. For Butelgut, steep loss of EIR reduction occurs for ADIs with effective ages above 3, consistent with a malaria development period equivalent to 2 gonotrophic cycles, and with high per cycle mortality (Figure 33 and Figure 34). Comparing results for Kankiya with those for the average data set, the profiles are almost identical for ADIs with effective ages up to four, consistent with the results in Figure 24 for a malaria development period equivalent to 3 gonotrophic cycles. Thereafter, EIR reduction is lost less quickly with increasing ADI effective age for Kankiya than for the average data. This profile is consistent with Kankiya's lower per cycle mortality rate compared to the average value (Figure 33 and Figure 34).

**Figure 47 Geographic sensitivity**  
 Comparison of reduction in infectious bites and selection coefficients for CIKI and ADIs using an average data set and the four geographic data sets [51] from which it is calculated.



For the selection coefficient, the primary drivers appear to be the natural mortality (Figure 33 and Figure 34) and human feeding propensity (Figure 31). For both these parameters, higher assumed values give lower selection coefficients and hence better resistance management. Background mortality has more impact for higher ADI effective ages, whilst human feeding propensity has more impact on selection coefficients with CIKI and lower ADI effective ages. The geographic sensitivities are consistent with these results. Butelgut with the highest non-human feeding propensity and the highest per-cycle background mortality has the lowest selection coefficient for all insecticides. Namawala has low non-human feeding propensity, which is most significant for CIKI and low ADI-effective ages,



giving a higher than average selection coefficient for CIKIs, falling with increasing ADI-effective age as the impact of high background mortality increases. Kankiya and Kaduna have very similar background mortality, and very similar selection coefficients for ADI effective ages above 2-cycles. Kankiya has much higher human feeding propensity, and consistent with this, has lower selection coefficient for CIKI and the lowest effective-age ADIs, where this difference has most effect.

Analysis of the individual data sets thus generates results consistent with our analysis using the average data set. Use of the average values therefore appears to be an appropriate simplification for the purpose of generating generalisable results.

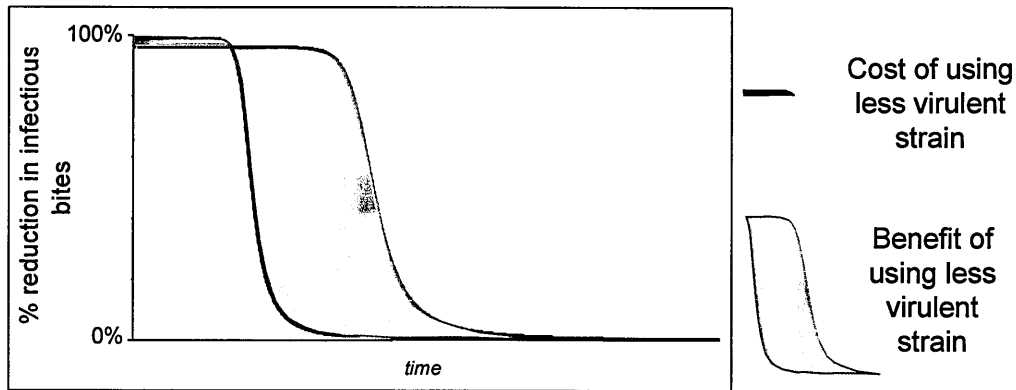
### **3.7 Discussion**

Our results predictably show a trade-off between the levels of transmission reduction accessible in a susceptible population, and the speed of spread of resistance. In general, earlier-acting insecticides give the greatest reduction in EIR, but also exert the strongest selection for resistance. Early gains are therefore paid for by shortening the useful life of the product (Figure 48). The importance of the initial level of transmission reduction depends on many factors, such as the EIR before treatment, the prevalence of malaria infection in the human population, other interventions used in conjunction with the LLA, budgetary constraints, and availability of alternative interventions when a product is lost to resistance. We therefore cannot offer a simple mathematical optimum for the trade-off between initial EIR reduction and long term resistance management, however, for any given context there are clearly substantial benefits to employing insecticide action, coverage and delivery methods designed to maximise the resistance management benefits achievable in combination with a given level of EIR reduction.

In general, it seems that for instant-kill, age-dependent, LLA insecticides, the best combinations of EIR reduction and low selection for resistance can be accessed at high coverage levels (Figure 19). Provided the same effective coverage values can be achieved

in each case, a pre-bite delivery method offers better transmission reductions than does post-bite, and allows better resistance management for a given level of transmission reduction (Figure 22).

**Figure 48 Trade off between initial levels of control and product useful life**



The natural mortality rate per cycle has a significant effect on EIR in the absence of any intervention, but makes little difference to the proportionate reduction in EIR possible using ADIs with effective ages in the most useful range. Selection coefficients are however reduced by higher background mortality per cycle, for all insecticides. Contexts with high natural mortality per cycle therefore offer good opportunities to access good resistance management in combination with good transmission control. High per cycle mortality can be the result of high per day mortality rates and/or long gonotrophic cycle lengths.

With high coverage and pre-bite delivery, comparable transmission control is offered by all ADIs with effective ages up to one cycle higher than the number of cycles required for a *Plasmodium* infection in a mosquito to develop to infectiousness (Figure 24). ADIs with effective ages 1 cycle less than the *Plasmodium* development period may offer transmission reduction almost equal to that provided by a conventional instant-kill insecticide. Resistance management improves with increasing effective age, so optimum combinations of transmission reduction and resistance management are in general offered by ADIs with effective ages between one cycle less and one cycle more than the *Plasmodium* development period. Locations with longer *Plasmodium* development periods offer scope to achieve high reductions in EIR using higher effective age ADIs

and hence generating low selection for resistance, and therefore offer opportunities to maximise the resistance management benefits of ADIs. This is particularly the case if a long *Plasmodium* development period is combined with high natural per cycle mortality, as is the case for the Namawala geographic data set (Figure 47).

Costs of resistance less than those observed in a natural system [59] can make resistant mosquitoes less fit than susceptible mosquitoes exposed to a 4 cycle effective age ADI at 80% pre-bite coverage, an intervention offering a 95% reduction in EIR in a susceptible population.

LLAs which generate lower or zero mortality in mosquitoes without a *Plasmodium* infection can greatly reduce selection for resistance, without compromising transmission reduction (Figure 27). Such products have the potential to be evolution proof if resistant phenotypes incur even very small costs of resistance (Figure 30) and should comprise a target for LLA product development.

The PM model indicates a very rapid spread of resistance in populations subject to high coverage of a conventional instant-kill insecticide, with >90% resistant phenotypes within a year. Our primary interest is the comparative timing of spread of resistance for different insecticides, but it is worth considering whether the values generated for CIKIs are plausible. Firstly it is important to remember that we are comparing the spread of resistance once a resistance allele is present in the population. Observed time to spread of resistance in the field necessarily includes any time required for resistance to arise. Nonetheless, dieldrin resistance has been observed in some field locations at >90% of the population after just two years of spraying [72]. Dieldrin is a particularly relevant example, because it is unusual in having virtually no spatial or contact repellancy [20]. Standard measures of resistance do not capture behavioral avoidance such as outdoor resting or feeding, or simply moving away from treated surfaces before acquiring a fatal dose, whereas the PM reflects the spread of all heritable resistance mechanisms. The most widely used conventional insecticides, DDT and pyrethroids all have varying levels of

spatial and contact repellancy, the lower selection pressure generated as a result (see section 4.5.2) compared to our assumptions, and the failure to monitor resistance expressed as avoidance behaviors would both lead to a lower observed rate of spread of resistance than that presented by our model.

Our models do not attempt to capture feedback between EIR reduction and the prevalence of malaria in the human population. This relationship is complex and location specific, and beyond the scope and requirements of this analysis. Even with an initial EIR below 1 in an untreated population, for example, reduction in EIR would not be expected to map directly to a proportionate reduction in malaria prevalence. Assuming that the probability of a susceptible individual becoming infected in a given period of time varies proportionately with the reduction in EIR, the proportion of susceptible individuals in the population would be expected to change, so that the number of infections per infectious bite might increase as the number of infectious bites declined. Our sensitivity analysis (Figure 36) shows that variance in the absolute value for the prevalence of infectious human hosts does not materially affect the proportionate reduction in EIR compared to an untreated population. However, if the value changed over time (which is, after all, the purpose of the intervention), the relevant comparison would be between EIR with an intervention and the reduced malaria prevalence and the EIR with the original malaria prevalence and no treatment. So for example, if sustained use of an intervention resulted in a 30% reduction in the prevalence of malaria in human population, our chosen measure of control, RAIB, would change by  $-0.3(1-RAIB)$ . Clearly the effect of such a change would be limited for CIKIs and early effective age ADIs which offer large reductions in EIR and consequently have RAIB values close to 1.

Our sensitivity analysis indicates that the values chosen as our measures for transmission reduction and resistance management give results which are robust to variation in parameters not directly involved in the interactions being assessed, consistent with our

expectations when choosing to formulate our key results as comparative rather than absolute values.

### 3.8 Conclusions

Our results indicate that LLA insecticides do offer good potential for achieving useful transmission reductions comparable with those available using conventional instant kill insecticides, whilst exerting much lower selection pressure in favour of the spread of resistance.

The optimum combinations of transmission reduction and resistance management can be accessed using delivery methods which provide high coverage, and, for instant-kill ADIs, a pre-bite delivery method. Given this, the most useful ADIs are those with effective ages within 1 cycle of the time required for *Plasmodium* to mature and become infectious in a mosquito host.

Locations best suited to maximising the benefits of ADIs are those with mosquito populations with high per cycle natural mortality and long *Plasmodium* development cycles. Vector species with higher propensities to feed on non human hosts also offer better resistance management in combination with a given level of transmission reduction.

In all contexts, differential mortality between *Plasmodium* infected and uninfected mosquitoes, with uninfected mosquitoes experiencing reduced or absent mortality, gives better resistance management for a given level of transmission reduction and, at high coverage values, this is achieved at minimal cost in terms of transmission reduction. Very small costs of resistance can make ADIs with this characteristic completely evolution proof.

Our results indicate that LLA insecticides offer great potential for transmission control using products which will not rapidly lose their utility to the spread of resistance.

## 4 Chapter 4 Fungal biopesticide LLAs

### 4.1 Introduction

Entomopathogenic fungi have a characteristic pattern of infection followed by an initial period of growth before generating mortality in an insect host. This delayed mortality means that such fungi offer potential to exploit the LLA concept [73]. Here we assess which of the wide range of possible virulence characteristics for fungal biopesticides can best realise this potential.

Naturally occurring strains of two fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, are already in commercial use for agricultural applications and have been shown to infect and kill mosquitoes in laboratory and field settings. Fungal spores can be picked up by mosquitoes following contact with treated surfaces, and so could be used against mosquitoes in indoor residual spray (IRS) programmes, or delivered via traps, curtains or netting [16,18,74-77].

A wide variety of mortality schedules can be induced in *Anopheles* by entomopathogenic fungi [17]. In some cases, all mosquitoes can be killed within a few days; in others, background mortality rates can be barely altered. This virulence variation depends on isolate [18], dose [78] and malaria-infection status [16,78], see also [79]. Lethality can also be increased by genetically modifying fungal isolates [80-82].

If fungal entomopathogens are to realize the potential of the LLA approach to sustainable malaria control, candidate biopesticides need to be chosen which balance reductions in parasite transmission (maximized by high fungal virulence) with resistance management (maximized by low fungal virulence). Here we use our LLA model to ask which virulence phenotypes best achieve this balance. The intention is to guide the development of target product profiles. The possible efficacy of fungal biopesticides in IRS campaigns is compared with that of pyrethroid-based insecticides now in widespread use. Pyrethroids

are highly lethal if contacted by a mosquito, but they also have a strong excito-repellency effect, which can drive away mosquitoes before they receive a lethal dose [20,83,84]. There is evidence that fungal spores do not repel mosquitoes [19], raising the prospect that, for IRS, fungal biopesticides might more effectively reduce transmission than pyrethroid-based technologies currently in use.

Our results show that fungal biopesticides which generate high rates of mortality at around the time mosquitoes first become able to transmit the malaria parasite offer potential for large reductions in transmission while imposing low fitness costs. Strains which have high virulence in malaria-infected mosquitoes but lower virulence in malaria-free mosquitoes, offer the ultimate benefit in terms of minimising selection pressure whilst maximising impact on transmission. Exploiting this phenotype should be a target for product development. For indoor residual spray programmes, biopesticides may offer substantial advantages over the widely used pyrethroid-based insecticides, not only offering substantial resistance management gains in the long term, but also providing greater reductions in transmission before resistance has evolved. This is because fungal spores do not have contact irritancy, reducing the chances that a blood-fed mosquito can survive an encounter and thus live long enough to transmit malaria.

## **4.2 Model Assumptions**

We use the FCM and PM developed for analysis of LLA insecticides and detailed in sections 3.2 and 3.3. The assumptions underpinning the FCM and PM therefore apply to this analysis, with the following amendments and additions.

All model parameters are age-independent, apart from incremental mortality from fungal biopesticide infection which varies according to the number of days since infection.

Mosquitoes are assumed to contact the CIKI or biopesticide when resting after biting a human host, reflecting an application method essentially consistent with IRS. Avoidance

behaviour such as outdoor feeding and outdoor resting is not reflected in the coverage values for susceptible mosquitoes since it comprises a method of resistance.

### **4.3 Parameter Values**

The baseline values used in the analysis are summarized in Table 6 and Table 7 (Section 3.4)

### **4.4 Analysis**

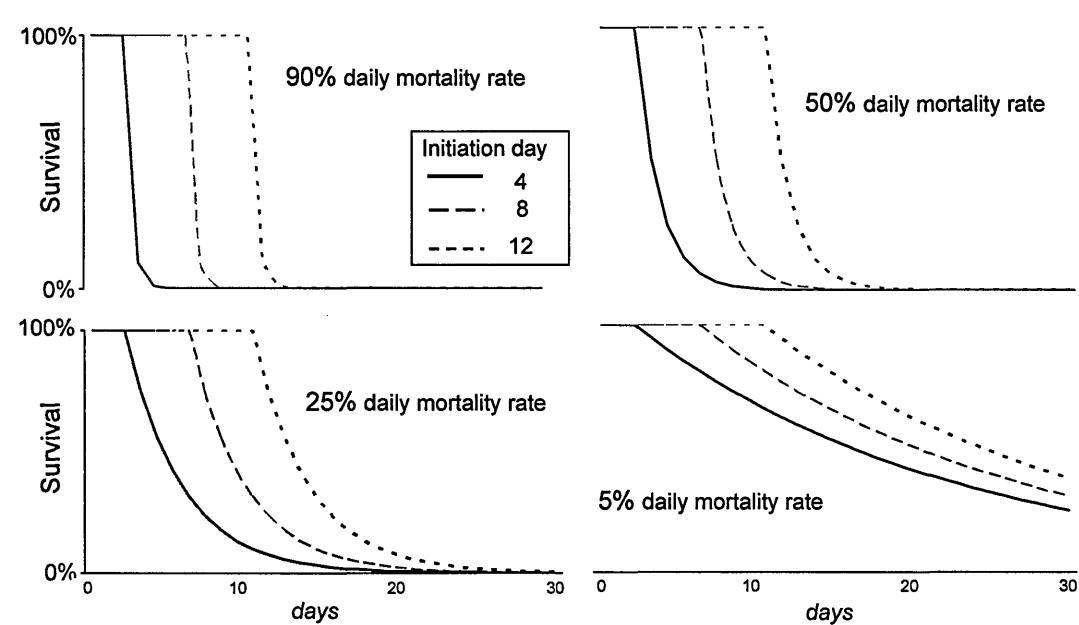
A number of fungal strains have now been tested in laboratory mosquito populations, and a wide range of mortality characteristics have been observed around the basic pattern of initial fungal growth and development followed by an increase in observable mosquito mortality [16,18,77,78,85]. This suggests that most virulence profiles are potentially available, and in a search for generalisable results we therefore use highly simplified fungal virulence characteristics, defined by two parameters, ‘initiation day’, the time from infection to the onset of fungus-induced mortality, and the daily mortality rate from that point. A number of illustrative survival curves generated from these parameters are shown in Figure 49.

Fungal biopesticides can also impact mosquito feeding propensity and flight capacity in the days before mosquito death [18]. A mosquito which no longer attempts to feed or to lay eggs is effectively dead from the perspectives of fitness and disease transmission. For the purpose of the model therefore, ‘mortality’ encompasses cessation of feeding and reproduction, as well as actual death.



**Figure 49 Illustrative survival curves for a range of simple virulence mortality assumptions**

Survival curves illustrating mortalities defined by two simple virulence parameters. For each illustrated pair of values, mortality is zero until the specified initiation day, and is thereafter maintained at the indicated fixed daily mortality rate. Initiation day and mortality rate are the two parameters used to define the assumed incremental mortality generated by a given biopesticide infection, and referred to here as ‘simple virulence’.



**4.5 Results**

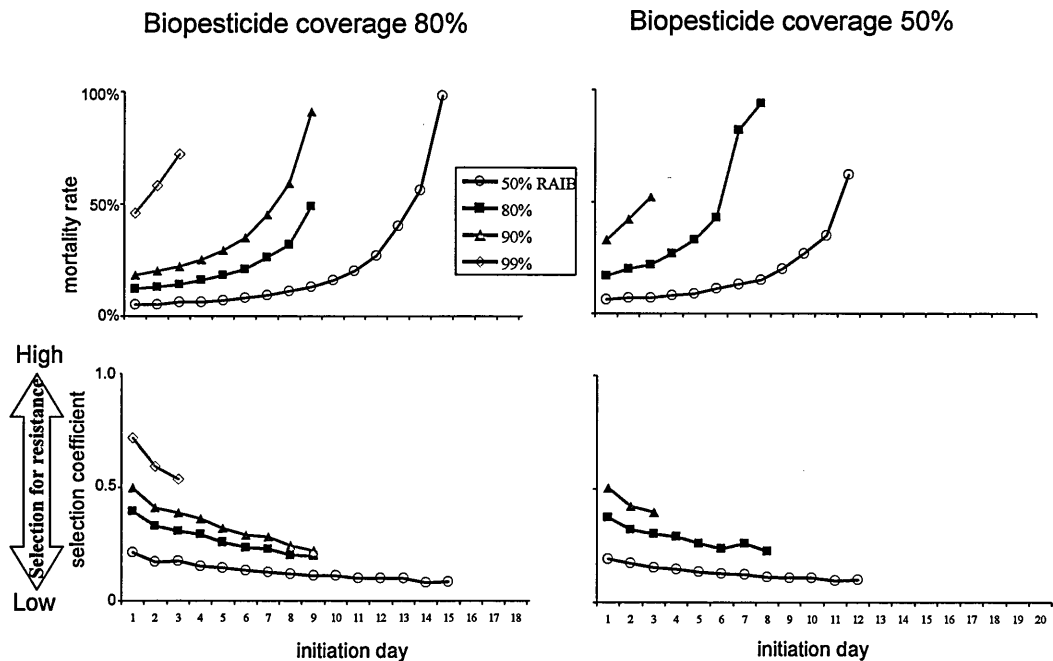
**4.5.1 Coverage and Virulence**

The proportionate reduction in EIR generated by use of a biopesticide is affected by fungal virulence and coverage (Figure 50). For a given level of coverage, similar levels of EIR reduction are achieved by various combinations of the two parameters used to summarize virulence (initiation day and mortality rate, Figure 49).

Unsurprisingly, the longer a fungus takes to initiate mortality, the greater the subsequent mortality rate has to be to maintain a given level of reduction in EIR. There are limits to the EIR reductions that can be achieved at low virulence and/or low coverage.

**Figure 50: Comparison of virulence characteristics and fitness costs associated with given reductions in EIR.**

Top panels show different combinations of values for initiation day (x-axis) and daily mortality rate (y-axis) which achieve the denoted reductions in EIR (RAIB). The mortality rate required to achieve a given RAIB increases for later initiation days, up to an initiation day beyond which the target RAIB cannot be achieved, at which point the plots stop. With 50% biopesticide coverage, no virulence parameter combinations achieve 99% RAIB. Bottom panels show the selection coefficients corresponding to the same set of virulence parameter values, eg, the 99% RAIB value plotted for initiation day 2 gives the fitness cost for susceptibility to a biopesticide with initiation day 2 combined with the mortality rate required to achieve a 99% RAIB. Higher selection coefficients indicate stronger selection pressure for resistance.



For equivalent reductions in EIR, selection for resistance is best minimized by high coverage with late initiation day, high mortality rate biopesticides. For example, the lowest selection coefficient associated with a 90% RAIB at 80% coverage is 21%, with day 9 initiation and a 91% mortality rate. At 50% coverage the lowest selection coefficient available in combination with 90% RAIB is 40%.

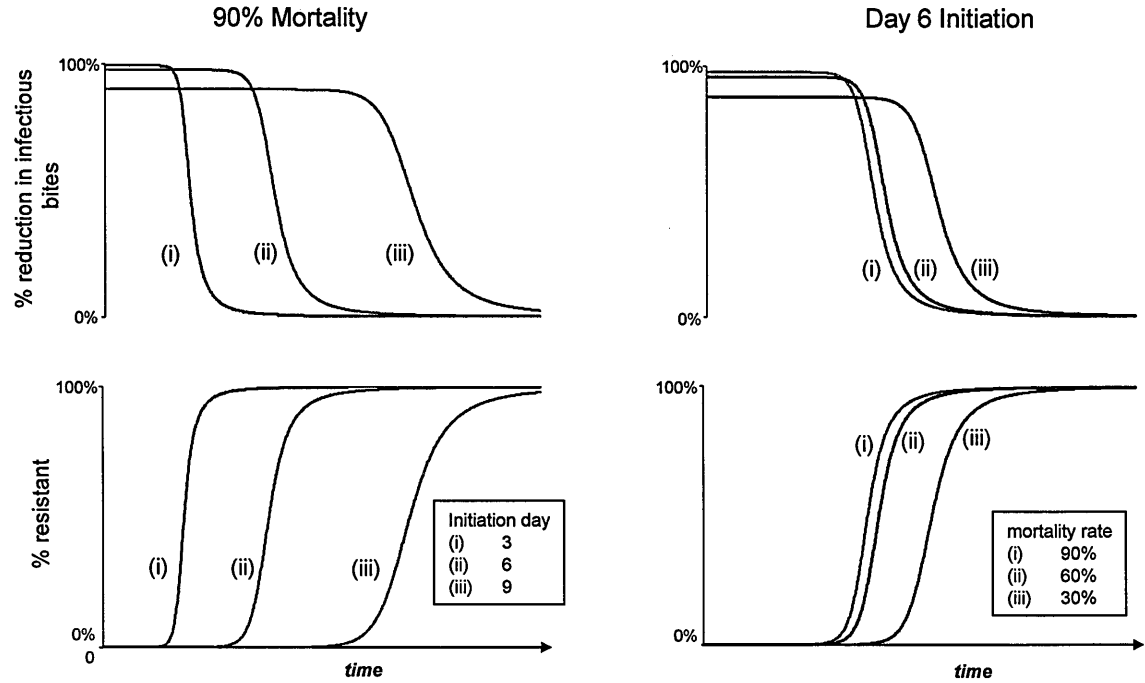
The temporal dynamics of EIR reduction and resistance evolution are shown in Figure 51. Predictably, more virulent biopesticides give better population-level reductions in EIR to begin with, but they then drive the evolution of resistance more rapidly. The speed of resistance evolution is more sensitive to the timing of mortality onset than to the incremental mortality rate.

The evolutionary dynamics and resulting pattern of control failure differ markedly for

different insecticides even when they give identical reductions in EIR for susceptible populations (Figure 52). Conventional instant-kill chemical insecticide (with coverage adjusted to achieve the same initial control as the biopesticides) fails first. The longest time to product failure is offered by a fungal biopesticide with relatively late mortality initiation, which then kills at a very high rate (Figure 52).

**Figure 51: Population level infectious bite rate and proportion resistant for populations exposed to different biopesticides**

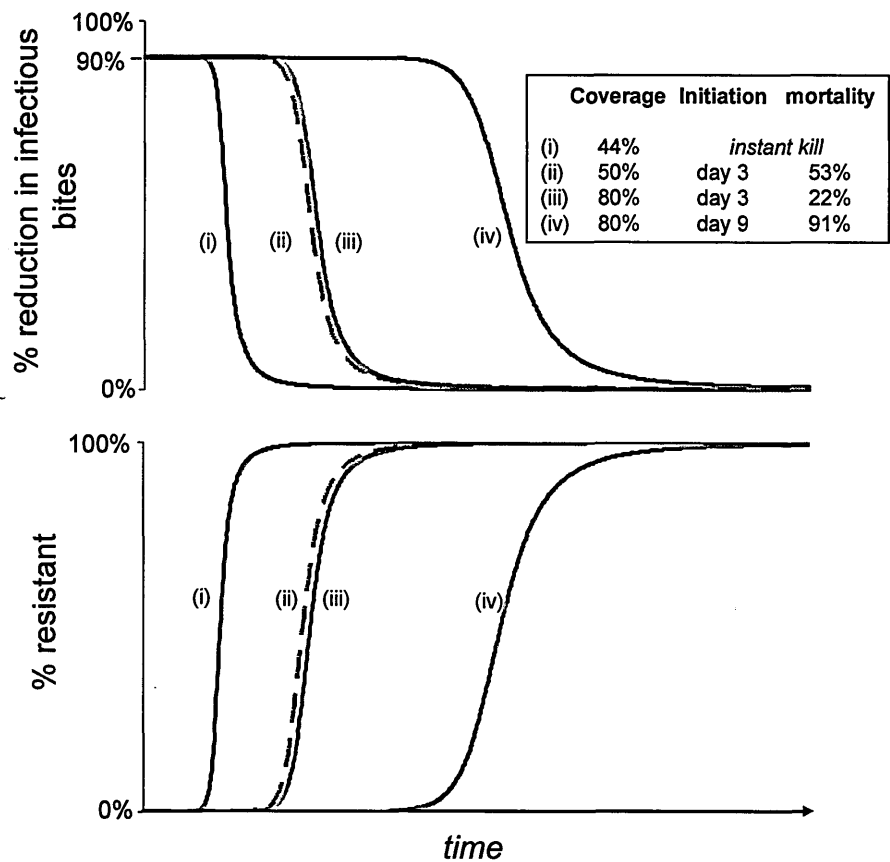
Top panels show the population reduction in infectious bites per unit of time for each of five different virulence combinations, and the change in this value over time with the spread of resistance to the treatments, shown in bottom panels. 80% coverage assumed throughout.



Clearly, the probability that a mosquito contacts and is affected by a vector-control treatment has a significant impact on both the reduction in EIR and reproductive success.

**Figure 52: Comparison of four interventions providing a 90% initial reduction in infectious bites**

Plots show the change over time in the proportion of resistant individuals (bottom panel) and the percentage reduction in population level infectious bites (top panel) for a mosquito population consistently exposed to one of four vector control treatments, all chosen to give the same 90% initial reduction in EIR.

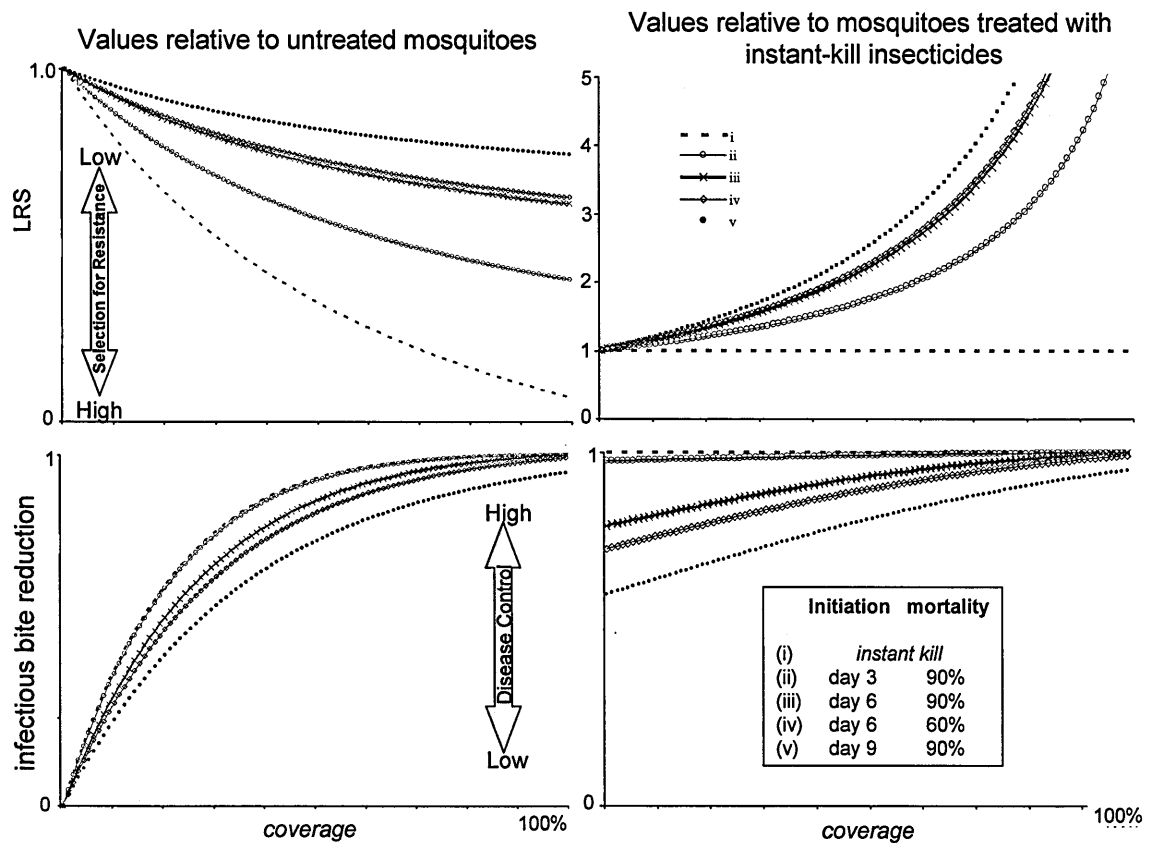


Reductions in EIR improve as coverage is increased, but the strength of selection for resistance also increases (Figure 53, left panels). This illustrates the predictable trade-off between the best transmission control, obtained at high coverage, and the best resistance management, obtained at low coverage. When compared to the currently available alternative, a conventional instant-kill chemical insecticide, however, the relative values for EIR reduction and resistance management with the biopesticides are maximized at the high coverage values which correspond to the best transmission control and the strongest selection pressures for resistance (Figure 53, right panels). Even a biopesticide with sufficiently high virulence to match the initial EIR reduction of instant-kill insecticides at the same coverage levels offers some benefit in terms of useful life (Figure 54). This is

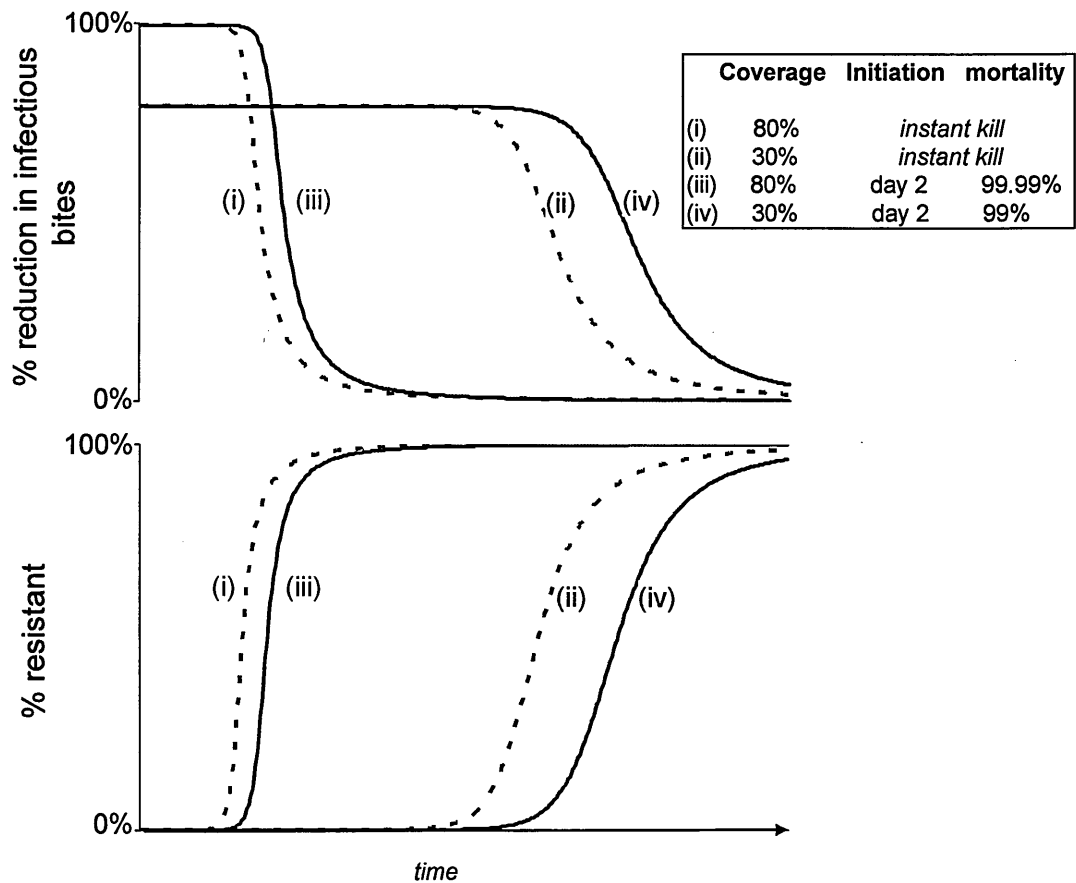
because fungus-infected mosquitoes are still able to achieve some reproduction before being killed, thus somewhat reducing the selection for resistance.

**Figure 53 Comparison of conventional instant-kill chemical insecticide and four biopesticides across a range of coverage values**

Lifetime reproductive success with interventions as a proportion of LRS for untreated mosquitoes (top left panel) and as a proportion of LRS for mosquitoes treated with an instant-kill insecticide (top right panel). Reduction in average infectious bites per mosquito lifetime with interventions, compared to the value for untreated mosquitoes (bottom left panel), 0 = no reduction in infectious bites, 1.00 = no infectious bites. Reduction in infectious bites with interventions vs untreated mosquitoes, compared to the reduction achieved using a conventional instant kill insecticide (bottom right panel), 1.00 means a reduction equal to that achieved by instant-kill insecticide.



**Figure 54: Comparison of resistance spread and consequent increases in infectious bites with instant-kill and fungal biopesticides**  
 Biopesticide virulence selected to give pre-resistance EIR reduction matching instant-kill pesticides at 80% or 30% coverage. Plots show the proportion of the population with resistant phenotypes, and the corresponding values for population-level reduction in infectious bites per unit of time compared to an untreated population.

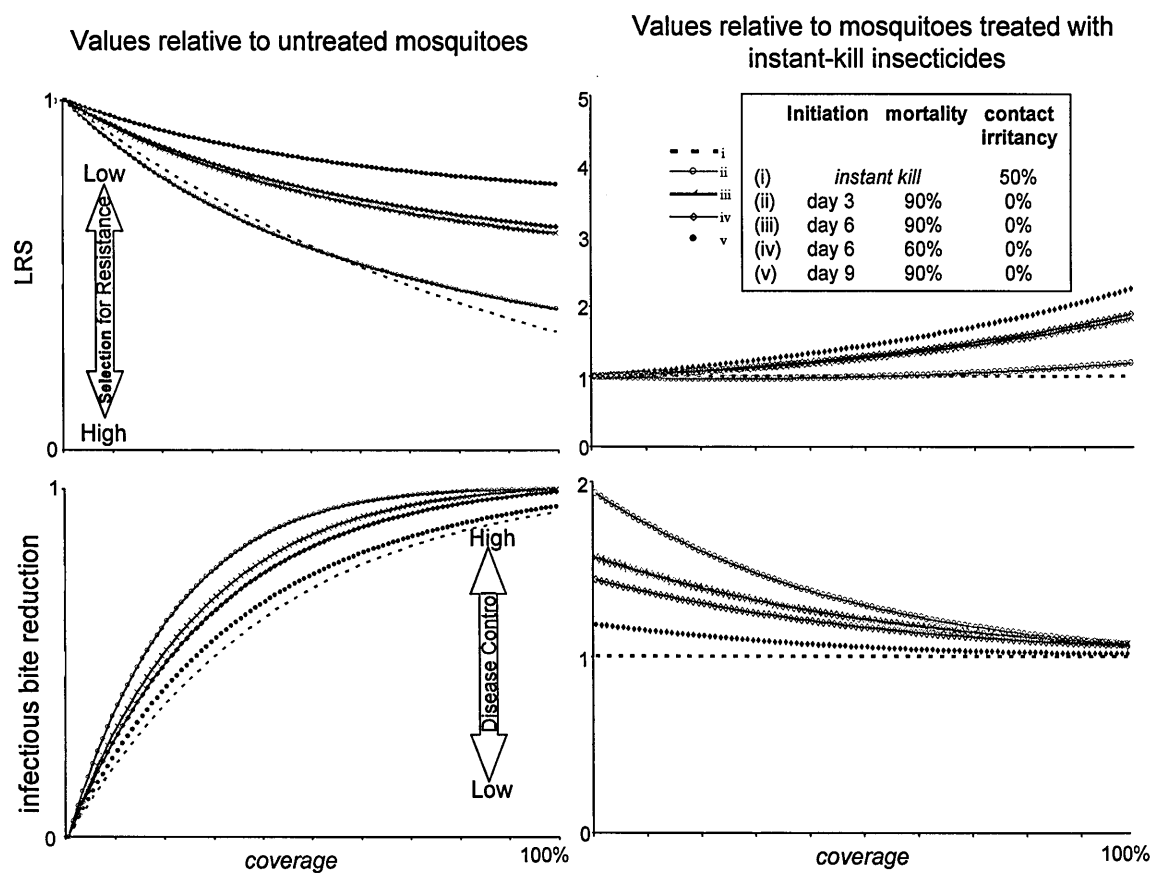


### 4.5.2 Repellancy

One of the most commonly used classes of conventional insecticides, pyrethroids, have high contact irritancy (also called excito-repellency), causing approximately 50% of mosquitoes contacting treated surfaces to be repelled without acquiring a harmful dose [20,83,84,86]. There is no indication of any repellency effects for the fungal biopesticides [19]. For IRS, if 50% of mosquitoes contacting the instant-kill insecticide are unaffected by it, then, for equivalent spray coverage, fungal biopesticides offer better reductions in EIR at all coverage levels, whilst maintaining selection benefits for all but the most virulent strain at the lowest coverage (Figure 55).

**Figure 55: Comparison between biopesticides and instant-kill insecticide with 50% contact irritancy, across range of coverage values**

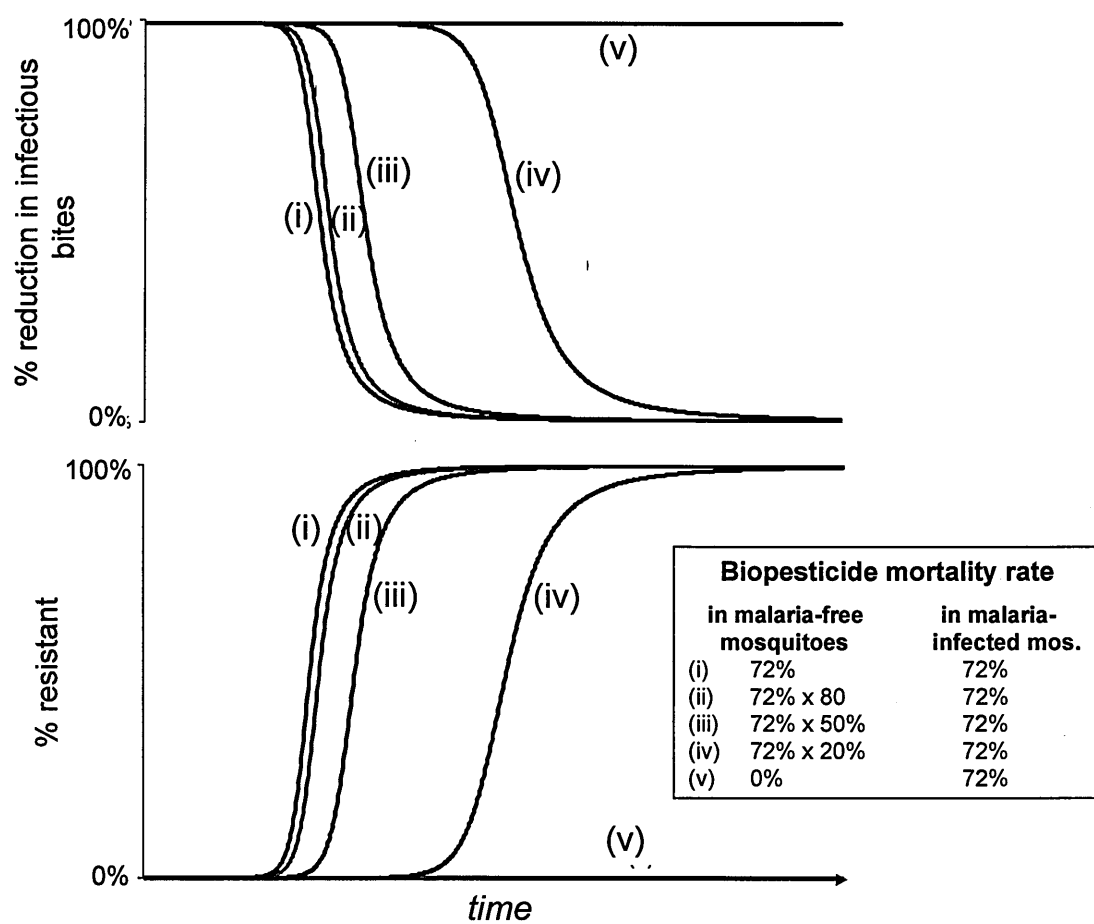
Lifetime reproductive success with interventions as a proportion of LRS for untreated mosquitoes (top left panel) and as a proportion of LRS for mosquitoes treated with an instant-kill insecticide with 50% contact irritancy (top right panel). Reduction in average infectious bites per mosquito lifetime with interventions, compared to the value for untreated mosquitoes (bottom left panel), 0 = no reduction in infectious bites, 1.00 = no infectious bites. Reduction in infectious bites with interventions vs untreated mosquitoes, compared to the reduction achieved using a conventional instant kill insecticide with 50% contact irritancy (bottom right panel), 1.00 means reduction in AIB equal to that achieved by instant-kill insecticide with 50% contact irritancy.



### 4.5.3 Malaria interactions

Some fungal strains have been shown to have higher virulence in malaria-infected mosquitoes than in those without malaria infection [16]. The trade-off between reducing EIR and resistance management is greatly reduced where fungal virulence is lower in malaria-free mosquitoes, with selection for resistance virtually eliminated if the fungus induces mortality exclusively in malaria-infected mosquitoes (Figure 56).

**Figure 56 Differential mortality in malaria-infected and malaria-free mosquitoes**  
 Comparison of speed of spread of resistance and consequent loss of transmission control for populations treated with one of five fungal biopesticides with differential mortality rates in malaria-infected mosquitoes. Plots show the proportion of the population with resistant phenotypes, and the corresponding values for population-level reductions in infectious bites per unit of time compared to an untreated population. The biopesticides all have day 3 initiation of a 72% daily mortality rate for malaria infected mosquitoes, giving an initial 99% reduction in infectious bites per time period.



#### 4.6 Discussion

Variation in the virulence characteristics of potential biopesticides offers scope for selecting strains targeted to provide desirable combinations of reduced transmission and resistance management. A number of virulence phenotypes can provide equivalent levels of EIR reduction (Figure 50), and in general high biopesticide-induced mortality rates commencing as late as possible offer better resistance management for a given level of pre-resistance EIR reduction (Figure 50 and Figure 52). There is nonetheless a trade-off between extending the time taken for resistance evolution to undermine efficacy of a pesticide, and the initial reductions in transmission (Figure 51). In general terms, and



consistent with our results in Section 3, more virulent fungal strains better reduce transmission initially, but at the cost of stronger selection for resistance, and consequently a shorter useful life.

Although high coverage offers scope to use less virulent fungal strains to reduce EIR, for given virulence parameters, higher levels of coverage also generate stronger selection for resistance, for both conventional and biopesticide interventions. Remembering that the biopesticides must be considered in relation to the best currently used approaches, it is interesting to note that in relative terms, the benefits of biopesticides *versus* conventional instant-kill insecticides are maximized at high coverage for both transmission control and resistance management (Figure 53).

The relative importance of initial control *versus* product lifespan depends on a large number of factors, including the availability of alternative replacement treatments, the meaning in terms of human morbidity and mortality of a smaller reduction in EIR at the outset, and the realities of public health budgets and other resources. The relative costs and benefits also change if the biopesticide is being considered for use as part of a combination treatment with other interventions [54,87,88]. There is, therefore, no simple mathematical optimum for the many possible virulence schedules; the many possibilities need to be considered in context. In so far as it can be done without compromising transmission control, however, it is clearly beneficial to choose the biopesticide that generates the lowest selection for resistance in a particular context. For resistance management, the aim should be to achieve high levels of coverage, allowing less virulent fungal strains to achieve a given level of control, and maximizing their resistance management benefits over instant-kill insecticides.

Even strains sufficiently virulent to match the transmission reducing characteristics of conventional instant-kill chemical insecticides at the same coverage levels still offer a small benefit in terms of the rate of spread of resistance (Figure 54). Such a resistance

management gain would be enhanced by any fitness costs associated with resistance [49] (see Section 3.5.5).

The conclusions presented here are independent of the method of resistance (e.g. metabolic or behavioural), provided it is genetically determined. It is assumed however that resistance is a binary quality, with mosquitoes either experiencing the full effects of a control measure, or remaining completely unaffected by it. The analysis of the speed of spread of resistance here thus assumes that susceptible mosquitoes experience infections with the specified virulence characteristics, and that resistant mosquitoes have no fungal mortality. In reality, it is more probable that a resistance/tolerance process would operate, with resistant mosquitoes still becoming infected, but experiencing a lower mortality rate than fully susceptible individuals. The spread of resistance would therefore effectively comprise a reduction in fungal virulence, rather than a complete loss of control. Considering the results presented in Figure 51, for example, this would mean that the spread of resistance to the highest virulence biopesticides, rather than comprising a steep function to complete resistance and total loss of transmission control, would move to the curves calculated for sequentially less virulent strains, as resistance converts high virulence strains to low virulence strains, offering even more beneficial resistance management possibilities. Future analyses could explore the impact of hypothetical resistance mechanisms that might operate with respect to conventional and fungal pesticides. The analysis presented here could also be extended to evaluate the impact of malaria infection on mosquito survival, fecundity and behaviour and variation in fecundity with mosquito age.

Certain widely used pyrethroid insecticides have high contact repellency, with studies suggesting that around 50% of mosquitoes landing on treated surfaces may leave before acquiring a fatal dose [20,83,84,86]. Whilst this potentially enhances the impact of pyrethroid-treated bed nets on transmission by deflecting mosquitoes away from protected

humans before they bite, for IRS it results in mosquitoes surviving to potentially transmit malaria in later feeding cycles [20]. Thus, for this group of conventional insecticides, the composite 'coverage' value at a given level of spray cover would be half that for biopesticides, and could never be greater than 50%. Comparing biopesticide results with a conventional insecticide, and assuming 50% contact repellency (Figure 55) across a full range of coverage values, fungi better reduce transmission than pyrethroid IRS, while still maintaining some resistance management benefits. This suggests that, for all spray coverage values, suitably virulent fungal strains might provide a better option for IRS-based vector interventions than contact-repellent pyrethroids. If only low levels of spray coverage are achievable, replacing repellent pyrethroids with high-virulence fungal treatments could significantly improve the achievable EIR reduction, without significantly increasing selection for resistance, which is in any case relatively weak at low coverage (Figure 55). Where high spray coverage is achievable, replacing pyrethroids with relatively low-virulence fungal treatments could give improvements in both transmission control and resistance management, since the relative fitness of susceptible mosquitoes would be potentially doubled.

The analysis shows that in all cases, having higher fungal-induced mortality in malaria-infected mosquitoes than in uninfected mosquitoes minimizes the fitness costs associated with a given reduction in transmission (Figure 56). The ideal biopesticide from the resistance management perspective would be one that had little or no impact on mosquitoes not infected with malaria, but was strongly virulent in malaria-infected individuals. This might be possible since malaria infection can impose significant metabolic and immunological challenges to mosquitoes [89-92]. There is only a minimal trade-off between transmission control and resistance management in malaria-linked incremental biopesticide mortality. By changing the fitness cost to the mosquito of malaria infection, pesticides working in this way might also exert selection in favour of vector resistance to malaria, further enhancing the transmission-control benefits from the

intervention. Strain selection or genetic modification should ideally target this trait. A further development of this principle would be fungal strains which specifically block development of the malaria parasite in the mosquito, or simply act as a delivery mechanism for anti-malaria interventions in the mosquito host ('paratransgenesis' [73,85]), with minimum survival or fecundity costs to the mosquito. It must be noted, however, that this potentially moves selection for resistance from the mosquito to the malaria parasite, which has so far proved extraordinarily adept at evolving its way out of trouble.

## **4.7 Conclusions**

This analysis shows that fungal biopesticides have the potential to significantly reduce EIR while imposing only weak selection for resistance. There is always a trade-off between the magnitude of the initial reductions in transmission and maintaining those reductions in the longer term. Given the severe human and economic consequences of malaria transmission, choosing an intervention which does not maximally reduce transmission at the outset requires very careful justification. However, the analyses presented here show that fungal biopesticides can offer equivalent or better reductions in transmission than existing interventions in both the short and long term. This is especially true where existing conventional chemical pesticides have high contact irritancy or resistance to them has already begun to spread. The theoretical analyses presented here should help define the vector mortality profiles required to maximize the sustained malaria control potential of fungal biopesticides, or indeed other novel biological or chemical insecticides.

## **5 Chapter 5 Case study, potential target for TDI development**

### **5.1 Introduction**

Molecular entomologist Kristen Michel, with Karajo Sprigg and others working in the Michel laboratory at Kansas State University, have identified a serpin, SRPN2, which negatively regulates the process of melanisation in the mosquito [21]. Melanisation is one arm of invertebrate immunity [93]. Using injection with dsRNA to deplete SRPN2, their experiments have revealed that the consequent upregulation of melanisation produces a time-delayed mortality schedule in affected mosquitoes, as well as reducing feeding propensity and fecundity. A chemical formulation achieving the same effect in a more easily delivered format therefore presents as a potential target for TDI development. We were asked to help investigate the disease control potential and resistance management characteristics of such a product.

Using experimental data provided by Michel et al, with an adapted version of our FCM model (chapter 3.2) we calculate values for the reduction in EIR and selection coefficient associated with use of a TDI producing effects equivalent to SRPN2 depletion, and compare these with values for theoretical LLAs and conventional instant-kill chemical insecticides.

### **5.2 Methods**

We were provided with survival, blood feeding, egg laying and hatch-rate data generated from a number of experiments using mosquitoes injected with dsRNA to produce SRPN2 depletion (hereafter "SRPN2KD"), alongside untreated controls and controls injected with green fluorescent protein (hereafter "injected controls").

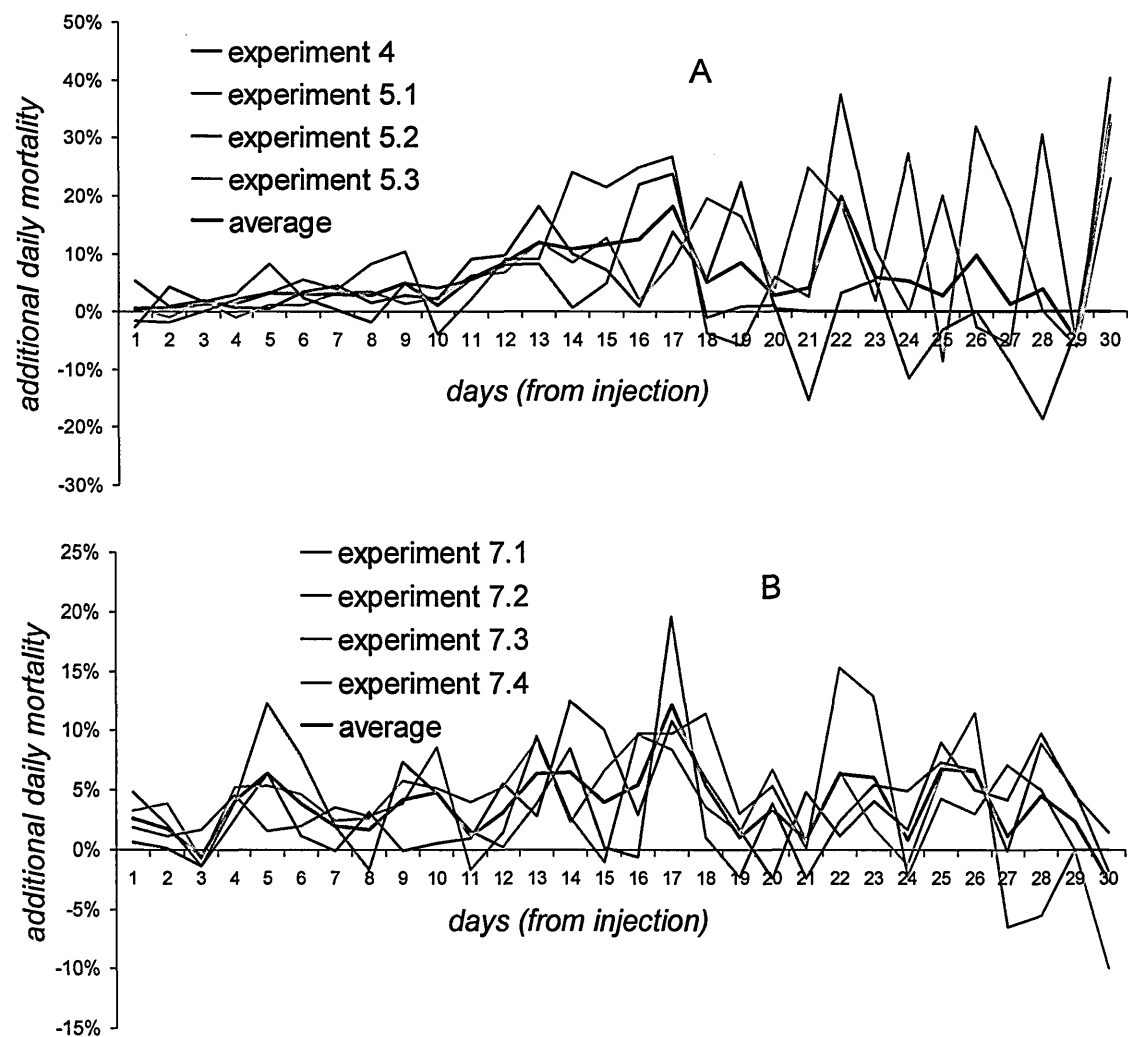
In order to evaluate the transmission reduction potential of SRPN2 depletion we need to identify the additional mortality experienced by SRPN2KD mosquitoes compared to that

experienced by control mosquitoes. We can then use these incremental mortalities in our FCM model in combination with background mortalities derived from field data. We need to do this rather than simply using the total laboratory SRPN2KD mortality figures because the LLA concept is predicated on the high mortality rates experienced by wild mosquitoes [49], and these are not reflected in laboratory mortality rates, where survival is generally much higher. The daily incremental SRPN2KD mortalities are the values required for  $\beta_x$  and  $\varepsilon_x$  in our FCM model (Table 4, p.30). Since we model 10 cycles of less than three days duration, we are not concerned with values beyond day 30.

We were provided with survival data for four replicate experiments for each of two feeding regimes. Mosquitoes were either offered daily opportunities to blood feed and oviposit, or were offered a blood meal only every fifth day. Since we are interested in the effect that a potential TDI would have in the field, we use the results from the daily bloodfeed experiments as these more closely resemble the natural situation than the five day blood feed experiments. Sensitivity analysis confirms (below) that this choice is not critical to our conclusions.

The SRPN2KD mosquitoes were injected with dsRNA to engender SRPN2 depletion. Since we are interested in the mortality arising from the effects of SRPN2 depletion but not those arising from the injection process, we make comparisons with the control mosquitoes injected with double-stranded green fluorescent protein, a neutral substance for this purpose. The differences between the daily mortality rates in the SRPNKD and injected control mosquitoes showed high variability between experiments, and rapid daily fluctuations within experiments (Figure 57). This was also the case for daily average values across all experiments within each feeding regime. Simple linear trend lines did not give a good visual match to the pattern of data.

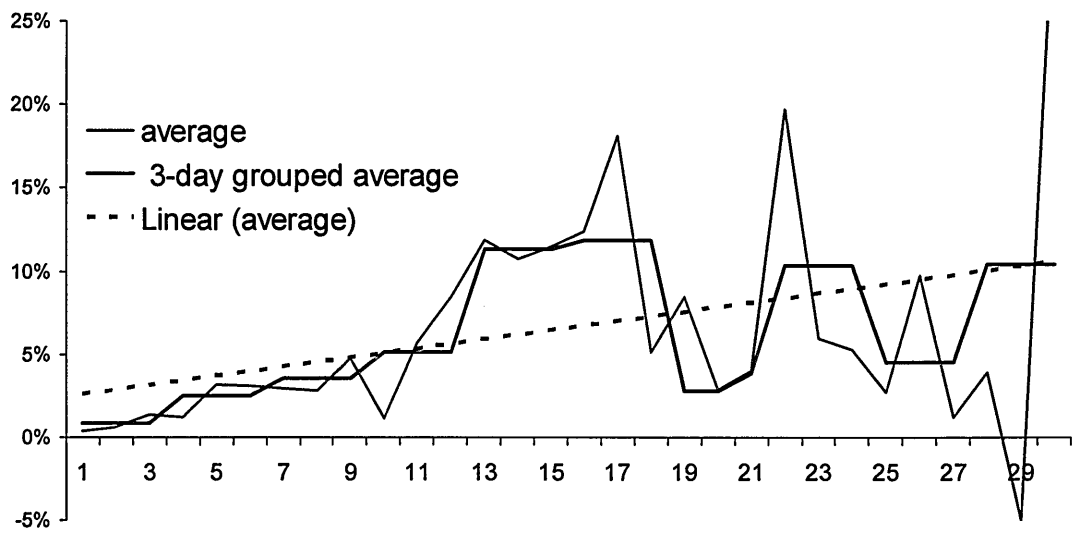
**Figure 57** Difference between daily mortality of SRPN2KD mosquitoes and that experienced by injected controls.  
Mortality differences calculated using the results from Michel *et al*, for daily blood fed (panel A) and 5 day blood fed (panel B) experiments, and corresponding average values. Experiment identification numbers are from the source data.



In order to capture time-related changes in incremental SRPN2KD mortality, therefore, whilst reducing arbitrary daily fluctuation, we process the data in the following way. For each experiment, for SRPN2KD and injected controls, we group the daily mortalities into three-day averages, for example we calculate the average values for SRPN2KD mortalities over days 1 to 3, days 4 to 5, and so on. Using the grouped averages as daily rates, so that the average for days 1 to 3 is used as the value in days 1 to 3, and so on, we subtract the grouped average mortalities for injected controls from those for SRPN2KD, giving grouped SRPN2KD incremental daily mortalities for each experiment. We use these results to calculate average daily values across all four experiments (hereafter “3 day averages”).

The averaging calculation ignores experiments which have no survivors (so when only three experiments still have survivors, averages are divided by three rather than four). As can be seen from Figure 58 this gives figures which appear to follow the pattern of the data, but with reduced fluctuation.

**Figure 58 Simple and grouped averages for SRPN2KD incremental mortality**  
Using data from Michel *et al*

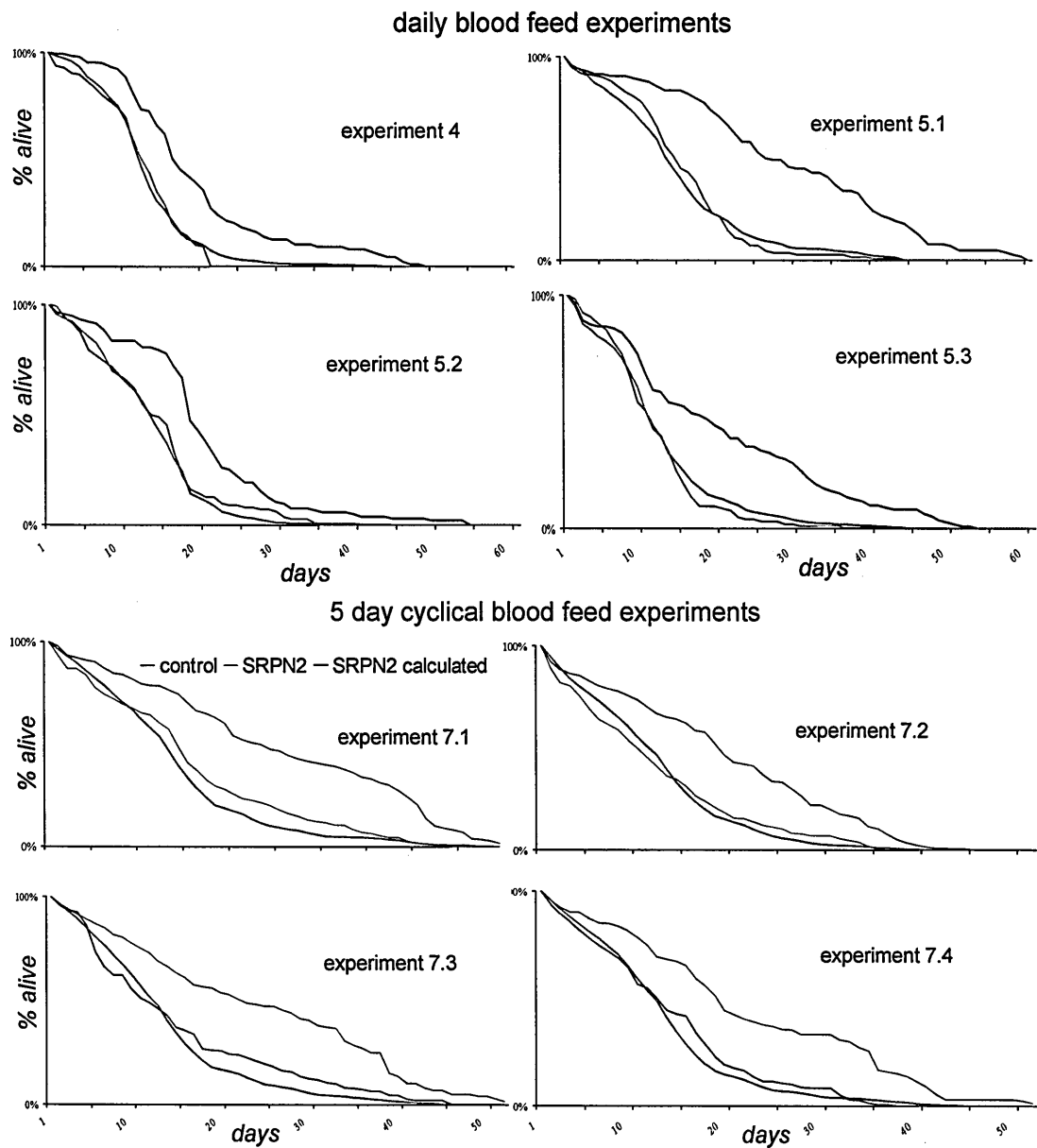


To check that these calculated incremental mortality values are an appropriate representation of the data, we add the 3 day averages to the unprocessed mortalities for injected controls in each experiment, and compare survival curves calculated using these values with the observed SRPN2KD survival curves. As can be seen from Figure 59 the results give a good visual fit in all cases, although predictably better with the daily blood feed experimental results on which they are based, than with the five day blood feed results.



**Figure 59 Survival curves using experimental data and calculated mortalities**

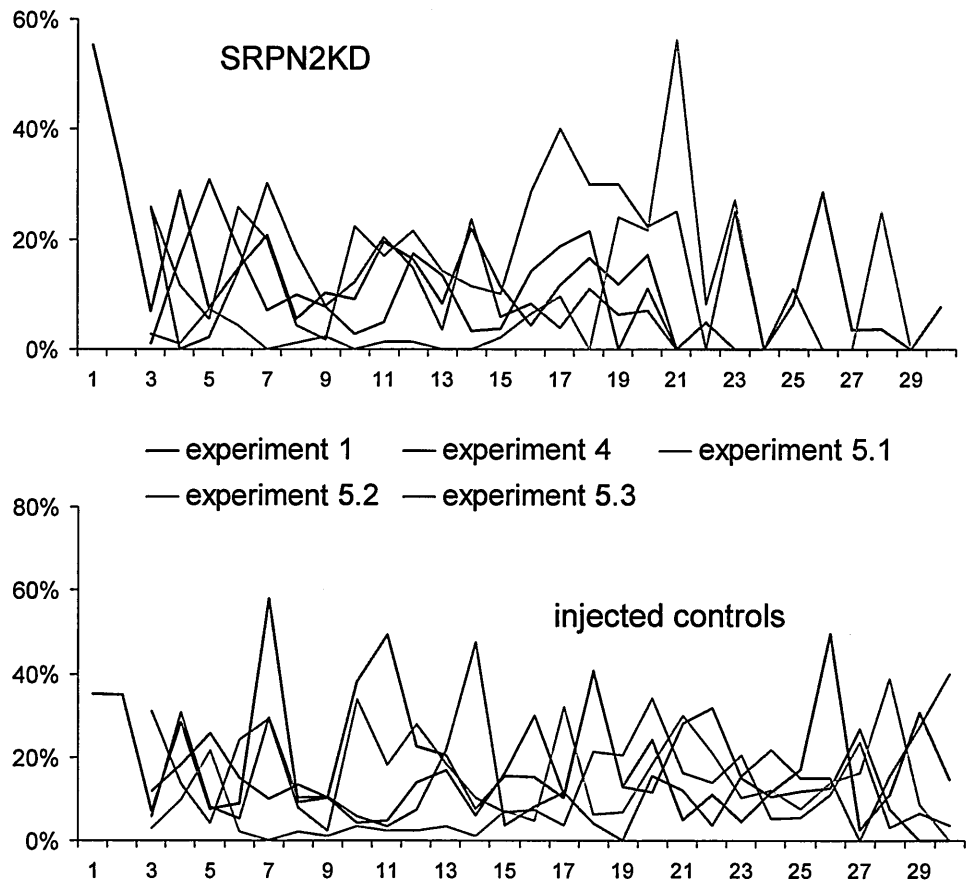
Each panel shows the survival curves for injected controls and SRPN2KD mosquitoes in a single experiment. The top four panels use data from experiments where daily blood feeds were offered, the bottom four use data from experiments which gave a blood feed on every fifth day. The SRPN2KD calculated plots show survival values produced using the injected control mortalities for each experiment plus the 3 day average incremental mortalities calculated from the daily blood feed experimental results. Panels are labelled with the experiment identification numbers provided with the original data sets. All experimental data provided by Michel *et al.*



In addition to incremental mortalities, we also need a value for the proportionate reduction in feeding propensity for SRPN2KD mosquitoes. Experimental data for the percentage of surviving mosquitoes which blood feed on any given day (Figure 60) is available for five experiments, including those used to calculate the 3 day average incremental mortality values. Simple comparison of the daily blood fed percentages is not adequate for definition of our required parameter value however, for a number of reasons. Firstly, the different

number of survivors on different days means that the daily blood feeding percentages should not be equally weighted, for example, periods in which only a small proportion of mosquitoes are still alive, and the percentage attempting to feed is an extreme high or low value will distort average values. Secondly, the daily results are very sensitive to the timing of feeding attempts. For example, consider a two-day period in which all surviving SRPN2KD and injected control mosquitoes attempt to blood feed exactly once. If feeding attempts for both categories of mosquito are similarly distributed in time, the daily blood feeding propensities will be the same for both groups of mosquitoes, indicating no difference in feeding propensity. However, if feeding attempts by SRPN2KD mosquitoes are 99% made on day 1, and by injected controls, 99% on day 2, the difference between the daily blood feeding propensities would be 98% on both days.

**Figure 60 Daily blood feeding propensities for SRPN2KD and injected controls.** Results from experiments (Michel *et al*) with blood feeds and ovipositing opportunities offered daily. Plots are the proportion of surviving SRPN2KD (top panel) and injected control (bottom panel) mosquitoes taking a blood meal each day.



To deal with these issues, instead of using the daily blood feeding propensities we consider the number of blood feeds per mosquito day. For example, if 100% of mosquitoes survive to day 1, with 60% of them feeding, and 50% survive to day 2, of which 30% feed, for an initial population size of  $N$ , over the two days we have  $N \times (1 + 0.5)$  mosquito days, during which there were  $N \times (1 \times 0.6 + 0.5 \times 0.3)$  blood feeds, giving  $N \times 0.75$  blood feeds in  $N \times 1.5$  mosquito days, an average of 0.5 blood feeds per mosquito day.

We calculate the average number of blood feeds per mosquito day for SRPN2KD and injected controls over the first 30 days of each experiment (i.e. over the period of interest for our model). For each experiment we then calculate the difference between these values for SRPN2KD and injected controls as a proportion of the injected control values, giving the proportionate difference in blood feeding propensity for SRPN2KD *versus* injected controls. The average of these values across all 5 experiments is 24%, and we use this as our base reduction in feeding propensity for TDI contaminated mosquitoes in our model. The feeding propensity values vary substantially between experiments, however, and we cannot know which set of experimental results correspond most meaningfully to conditions in the field, nor whether field conditions would simply produce all the possible outcomes at different times, we therefore bracket this average value with evaluations using the smallest (7.5%) and largest (59%) single experiment values for reductions in feeding propensity.

The FCM model (chapter 3.2) assumes that all mosquitoes which survive long enough to do so will attempt to feed, and then go on to lay eggs, in each gonotrophic cycle during a lifetime. We therefore have to amend the model to incorporate the possibility of mosquitoes missing a feed during a given sporogonic cycle.

We create a new parameter,  $\Lambda_l$ , feeding propensity for mosquitoes with a TDI status of  $l$ , with  $l = 0$  for mosquitoes uncontaminated with a TDI, and otherwise  $l$  is equal to the number of cycles since contamination with the TDI, as defined in Table 4, p30. Mosquitoes with no TDI continue to attempt to feed in every cycle so  $\Lambda_0 = 1$ . We then

adjust the probabilities of survival, giving an infectious bite and egg-laying to reflect the possibility that a given proportion of mosquitoes will not feed or lay. It is assumed that mosquitoes which do not feed or lay in a given cycle will still experience the daily background mortality associated with a period of time equal to the length of one gonotrophic cycle.

The number of infectious bites given in cycle  $i$  by mosquitoes with TDI status  $l$ , will be reduced in direct proportion to the reduction in feeding propensity. Equation (13), p.64, is therefore revised to

$$I_i = \frac{\sum_{m=D}^{i-1} \sum_{l=0}^{i-1} \left( \Lambda_l (q_{i,m,l,2} v_{i,m,l} + q_{i,m,l,3} v_{i,m,l}) \right)}{V_i} \quad i > D$$

The probability that a mosquito starting cycle  $i$  with malaria status  $m$  and TDI status  $l$  will not attempt to bite any host, nor to lay eggs, and will survive to the start of cycle  $i+1$  is the probability that a mosquito with TDI status  $l$  will not attempt to feed, multiplied by the probabilities of surviving a number of days equivalent to the time spent host seeking, resting and searching for a laying site. Since this model adaptation is specifically for the evaluation of a TDI we can assume that the probability of contacting a conventional instant-kill insecticide is zero, so  $k_{1,i} = 0$  and  $k_{2,i} = 0$ . The probability of surviving a cycle without feeding or laying is therefore equal to the probability of surviving the cycle with feeding and laying, adjusted to remove the specific feeding-related mortalities,  $a_1$  and  $a_2$ . The probability of surviving to the start of cycle  $i$ , with an existing malaria infection, given by equation (18), p.69, is therefore reformulated as

$$v_{i,m,l} = v_{i-1,m-1,l-1} \left( \left( q_{i-1,m-1,l-1,1} + q_{i-1,m-1,l-1,2} + q_{i-1,m-1,l-1,3} \right) z_{i-1,m-1,l-1} \left( \Lambda_{l-1} + \frac{1 - \Lambda_{l-1}}{(1-a_1)(1-a_2)} \right) \right)$$

$i > 1 \quad m > 1 \quad l > 1$

Non-feeding mosquitoes cannot acquire a new malaria infection, so equation (17), p.69, can be amended to

$$v_{i,0,l} = v_{i-1,0,l-1} \left( (q_{i-1,0,l-1,1} + q_{i-1,0,l-1,2} + q_{i-1,0,l-1,3}) z_{i-1,0,l-1} \left( \Lambda_l + \frac{1 - \Lambda_l}{(1 - a_1)(1 - a_2)} \right) - M q_{i-1,0,l-1,3} z_{i-1,0,l-1} \Lambda_l \right)$$

$i > 1 \quad l > 1$

and equation (16), p.68, to

$$v_{i,1,l} = v_{i-1,0,l-1} q_{i-1,0,l-1,3} (1 - \Lambda) M z_{i-1,0,l-1}$$

$i > 1 \quad l > 1.$

The probability of surviving through cycle  $i$  with an existing TDI infection, given by equation (19), p.70, is revised to

$$s_{i,m,l} = \left( \sum_{h=1}^3 q_{i,m,l,h} \right) z_{i,m,l} \left( \Lambda_l + \frac{1 - \Lambda_l}{(1 - a_1)(1 - a_2)} \right)$$

$l > 0.$

Only mosquitoes which blood-feed are assumed to lay eggs in any given cycle. The average number of eggs laid in cycle  $i$  by mosquitoes starting cycle  $i$  with malaria status  $m$  and TDI status  $l$ , equation (15), p.66, is thus revised to

$$f_{i,m,l} = L(1 - \theta) E_{1,m} E_{2,l} \left( \sum_{h=1}^3 q_{i,m,l,h} \right) \Lambda_l z_{i,m,l}$$

$l > 0$

We need an empirical estimate of fecundity for SRPN2KD to use for parameter  $E_{2,l}$ , the proportion of eggs laid per oviposition by mosquitoes contaminated with a TDI  $l$  cycles ago, compared to those laid by mosquitoes with no TDI contamination (Table 4, p.64). The experimental results include the number of eggs laid per bloodfed female and the subsequent hatch rates, for SRPN2KD bloodfed females and injected controls, for four feeding cycles (Table 21).

We use the number of hatching eggs per bloodfed female as a basis for comparing fecundity of SRPN2KD and injected controls. We calculate the relative numbers of hatching eggs per SRPN2KD female as a proportion of those laid by injected controls for each cycle, and use the average of these results, 0.40, as our fecundity adjustment factor.

We also produce sensitivity cases using the highest and lowest per cycle values, 0.53 and 0.32.

**Table 21 Summary of experimental reproduction data and calculation of relative SRPN2KD fecundity**

	SRPN2KD			injected controls			SRPN2KD relative fecundity
	Eggs /bloodfed female	hatch rate	eggs × hatch rate	Eggs /bloodfed female	hatch rate	eggs × hatch rate	
Cycle 1	31.57	52%	16.33	67.11	75%	50.45	0.32
Cycle 2	18.68	60%	11.12	46.89	72%	33.99	0.33
Cycle 3	20.54	76%	15.52	49.64	71%	35.04	0.44
Cycle 4	23.54	56%	13.29	37.87	67%	25.30	0.53
average							0.40

### 5.3 Results

Using the amended FCM model we calculate the reduction in infectious bites and the selection coefficient for a TDI with equivalent effects to SRPN2 depletion (hereafter “SRPN2 TDI”), assuming 80% coverage and other parameter values as defined above and in Table 6, p.80. Our results, summarised in Table 22, indicate that RAIB is very dependent on the SRPN2 TDI’s effect on blood feeding propensity. A TDI producing the same mortality effects as SRPN2 depletion, but no changes in fecundity or feeding propensity, offers a reduction in infectious bites of only 54%, unlikely to be sufficient as a stand-alone intervention. When reduced blood feeding propensity is considered in addition to increased mortality, however, infectious bite reductions range from 68% to 94%, the maximum value approaching the infectious bite reductions calculated for a conventional instant-kill insecticide at the same coverage levels. Reduced blood feeding propensity, however, also increases the selection coefficient because mosquitoes which do not feed do not lay eggs, increasing the relative fitness of resistant phenotypes, and this is exacerbated by the reduction in fecundity for those females which do produce eggs, so that an infectious bite reduction of 94% is associated with a selection coefficient of 0.49, and even the lowest reduction in feeding propensity, offering a 68% reduction in infectious bites,

generates a selection coefficient of 0.41, compared to 0.35 for a conventional instant kill insecticide at coverage levels providing the same infectious bite reduction.

With average feeding propensity, an 84% reduction in infectious bites is achievable, but with a selection coefficient of 0.46, almost as high as that for a CIKI giving the same transmission reduction.

**Table 22 Results summary for effects of SRPN2 depletion on malaria transmission and mosquito fitness**

Figures calculated using incremental mortality figures for SRPN2KD based on 3 day averages from daily blood feed data. Sensitivity results (a) using incremental mortality figures for SRPN 2KD based on 5-day averages from 5 day blood-feed data (b) using base case incremental mortalities with minimum and maximum values for relative fecundity

Results Summary SRPN 2KD	Assumptions		Results		
	Relative Feeding Propensity per cycle when 'infected'	Relative Fecundity per lay when 'infected'	Transmission reduction (proportionate reduction in infectious bites per mosquito lifetime)	Selection Coefficient	Selection Coefficient for conventional instant-kill insecticide giving same reduction in infectious bites
Using incremental mortality schedule derived from 3-day grouped averages from daily bloodfeed experiments					
No fecundity or feeding propensity adjustment	1.00	1.00	54%	0.13	
Minimum reduction in feeding propensity	0.925	1.00	68%	0.21	0.35
Average reduction in feeding propensity	0.76	1.00	84%	0.32	0.49
Maximum reduction in feeding propensity	0.41	1.00	94%	0.40	0.65
Min. reduction in feeding propensity + fecundity reduction	0.925	0.40	68%	0.41	0.35
Av'ge reduction in feeding prop. + fecundity reduction	0.76	0.40	84%	0.46	0.49
Max. reduction in feeding propensity + fecundity reduction	0.41	0.40	94%	0.49	0.65
Summary sensitivity results SRPN 2KD					
5 day grouped average mortalities (a)					
Minimum reduction in feeding propensity	0.925	0.40	64%	0.42	
Average reduction in feeding propensity	0.76	0.40	83%	0.46	
Maximum reduction in feeding propensity	0.41	0.40	93%	0.49	
Base case mortalities, fecundity sensitivities (b)					
Av'ge reduction in feeding prop. + fecundity reduction	0.76	0.32	84%	0.47	
Av'ge reduction in feeding prop. + fecundity reduction	0.76	0.53	84%	0.43	

Sensitivity analysis confirms that these results are robust to our choice of data. It can be seen from Table 22 that using incremental mortalities derived from the five-day blood feed experimental results, rather than from the daily blood feed data, does not materially change the results for either EIR reduction or selection coefficient. Using the maximum and minimum relative fecundity values for individual experiments also gives results consistent with our base case.

## 5.4 Discussion

The mortality produced by SRPN2 depletion offers levels of transmission control considerably lower than those available with CIKIs at the same coverage levels, but when reductions in feeding propensity are also taken into account, levels of transmission control comparable to those achieved using current CIKIs may be achievable. The potential for a SRPN2 TDI to be developed into a practical transmission control intervention is therefore dependent on its impact on feeding propensity in the field. A high degree of variation in the feeding propensity values was apparent in the experimental data, however, making this hard to predict. It may be that the stresses which increase background mortality in the field interact with the effects of SRPN2 depletion to give a consistently high reduction in feeding propensity. Alternatively, however, field conditions might minimise the effect of SRPN2 depletion on feeding propensity, limiting the transmission reductions achievable.

Reduced feeding propensity also reduces the fitness of susceptible mosquitoes, so the transmission control benefits it provides come at the cost of higher selection for resistance. Reduced fecundity in SRPN2KD mosquitoes further increases the strength of selection for resistance to a SRPN2 TDI and the results summarised in Table 22 indicate that a SRPN2 TDI would not achieve a combination of transmission reduction and low selection for resistance equivalent to those calculated in previous chapters for theoretical LLA insecticides. For example, we calculate (Figure 50, p.133) that an appropriately virulent fungal biopesticide could offer a 90% reduction in infectious bites combined with a selection coefficient of 0.21, and (Table 12, p.86) that a 4-cycle ADI could offer a 95% reduction in infectious bites combined with a 0.22 selection coefficient, whereas for a SRPN2 TDI we calculate selection coefficients between 0.41 and 0.49 for infectious bite reductions between 68% and 94%.

If its potential as an LLA is limited, then the performance of a SRPN2 depletion TDI needs to be compared to that of existing conventional insecticides simply as a potential new



transmission reduction tool. As discussed above, this is highly dependent on the reduction in feeding propensity produced by the TDI. If the largest feeding propensity reduction we consider was achieved in the field, a SRPN2 TDI could offer a 93% reduction in infectious bites, lower than the 99% calculated for a conventional instant-kill insecticide applied at the same coverage levels (Table 12, p.86), but better than the 87% calculated for an instant kill insecticide with 50% contact irritancy (Figure 55, p.138), assuming no contact irritancy for the new TDI. If the average or the lowest single-experiment feeding propensity reductions of 24% or 7.5% were achieved in the field, we calculate transmission reductions of 84% or 68% respectively, both lower than the transmission reductions offered by conventional instant-kill insecticides assuming the same (80%) coverage levels, and some very specific benefit, for example low cost, high persistence, high specificity or low toxicity, would presumably be required to make a SRPN2 TDI a candidate for commercial development in such case.

With the current data, we would conclude that a SRPN2 TDI would not offer the necessary combination of transmission reduction and resistance management benefits to make it a promising candidate for development as an LLA insecticide. It is not clear that it could offer consistently good transmission control in the field, but if it could, it would not be in combination with good resistance management.

## **6 Chapter 6 Modelling the spread of resistance – generation overlap matters**

### **6.1 Introduction**

Results from the sensitivity analysis of our LLA model (Section 3.6.7) show that an assumption of recessivity *versus* dominance for resistance alleles is important, but has much less influence on the speed of spread of resistance than the differences in selection coefficient between the various insecticides we evaluate (Figure 42). This is inconsistent with conventional wisdom in resistance management however, which assumes that, if resistance alleles are recessive, resistance will not spread, or will spread extremely slowly (eg. [94-96]), and that this effect outweighs that of selection pressure in determining the speed of spread of resistance. We here explore the cause of this disparity, and identify a key assumption which determines which paradigm applies in specific contexts.

Review of the literature reveals that the assumption of little or no spread for recessive resistance alleles is generally based on the use of a standard population genetics model (hereafter the ‘replacement model’ or ‘RM’), as described by John Maynard Smith in his classic text book “Evolutionary Genetics” [97]. The RM considers discrete time-periods during which generations are produced, develop to maturity, reproduce once and die. These assumptions match the realities of a semeloparous life history with seasonal reproduction, like that of the mayfly [98]. The breeding adults in populations of Anopheline mosquitoes comprise a mixture of ages and generations, with surviving adults which have previously reproduced, as well as newly mature adults, all contributing to the genotypes of new offspring. Hereafter we refer to this mixing of surviving adults from one

cohort with newly mature adults of a later cohort, as generation overlap. We show below, using comparison with our PM model, algebraic manipulation and numeric analysis, that for iteroparous populations with overlapping generations, such as anopheline mosquitoes, the RM significantly underestimates the speed of spread of initially rare recessive resistance alleles. Use of the RM in this context will therefore lead to misguided optimism about the predicted spread of resistance when resistance is recessive, and misplaced anxiety about the consequences of switching from functionally recessive to functionally dominant systems [95,99,100], as further discussed below..

## 6.2 Analysis

The RM is a standard population genetics model, [97] which tracks the spread of resistance alleles within a population through sequential discrete time periods. The model calculates the proportion of resistance alleles in the population at the beginning of each modelled time period. Offspring produced by the population in one period constitute the population at the beginning of the following time period, with proportions adjusted to reflect differential survival for resistant and susceptible phenotypes, and so on. Thus reproductive adults in one modelled time period are assumed to be entirely replaced by their offspring in the following modelled time period. This assumption is clearly appropriate for many combinations of life-history and time period. For many species however, including major disease vectors such as *Anopheles* mosquitoes, populations comprise multiple generations, all contributing to the genetics of the next generation of offspring. Although there are many details of such populations which the RM does not capture, for example population age-structure, we show here that, for initially rare recessive resistance alleles a primary source of the inconsistency between the RM and more detailed models is the RM's failure to capture simple adult survivorship between modelled time periods.

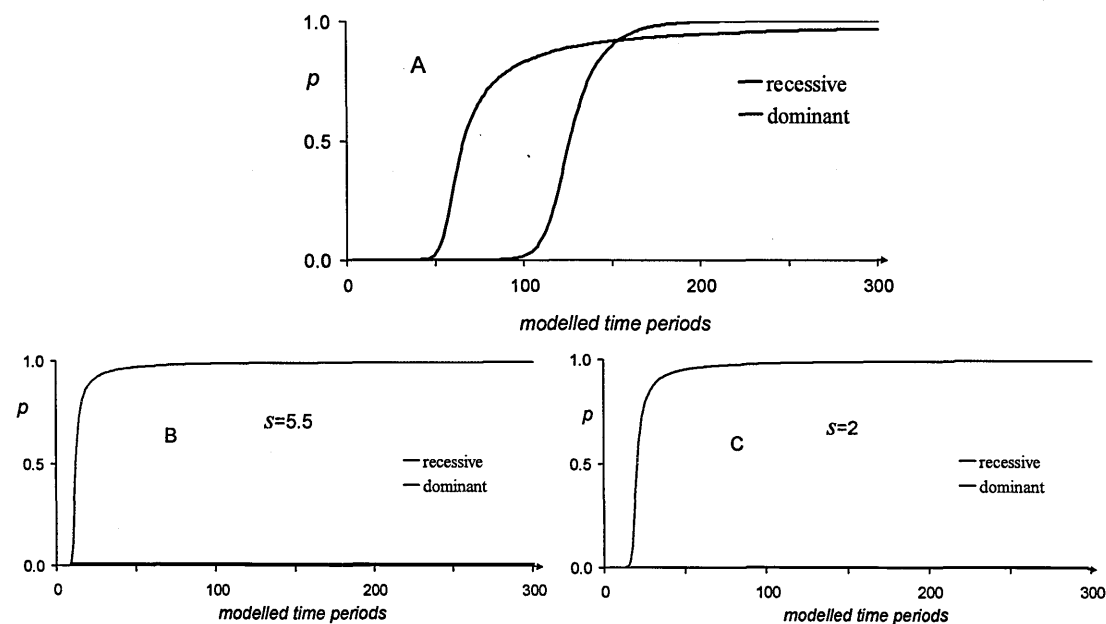
Our analysis, calculated using the PM (chapter 3), of interventions using a conventional instant-kill insecticide (CIKI) provides a helpful basis for comparison with the results of

the RM, as the mortality and reproduction values are the same for all mosquito ages, minimising the effects of age-structuring in the PM. It can be seen from Table 8 and Table 11 that resistance to a CIKI (assuming no cost of resistance) gives 3 times the probability of surviving through a gonotrophic cycle, and hence the opportunity to produce 3 times as many eggs per cycle. If we take account of survival between feeding cycles, the lifetime fitness of resistant phenotypes is 6.5 times that of susceptibles. Whereas it is easy to interpret the parameters of the RM with respect to populations which follow seasonal breeding patterns in which adults reproduce once and then die, it is difficult to define biologically meaningful data sets for the RM when considering populations in which multiple adult generations overlap and adults reproduce repeatedly. It is in essence the failure of the RM to properly capture this situation which lies at the heart of this analysis. We find however, that, as can be seen in Figure 61, whether we assume that the RM time periods equate to a feeding cycle, and use relative fitness for resistants of 3, so  $s = 2$ , or that the RM time periods somehow equate to an adult lifetime, and use relative fitness for resistants of 6.5, so  $s = 5.5$ , our conclusions are unchanged and, compared to the PM, the RM substantially understates the speed of spread of rare recessive resistance alleles. For both  $s = 2$  and  $s = 5.5$ , resistance has not spread after 10,500 modelled periods using the RM, whereas resistance alleles are close to 100% after 150 periods in the PM results. This is not the case if resistance is assumed to be dominant, the RM results in such cases actually showing somewhat faster spread of resistance than the PM. Note that the profiles of the results plotted from the PM shown in Figure 61 differ from those shown in chapters 3 and 4 primarily because they are the proportion of resistance alleles rather than the proportion of resistant phenotypes.

A number of assumptions in the PM would be expected to contribute to differences in results when compared to the RM. The PM captures age-structuring in the population, delay between the timing of reproduction and the time when adult offspring join the

population, mismatch between male and female genotypes, single mating at maturity, adult but not juvenile mortality and adult survival between modelled time-periods. The use of CIKI data eliminates age structure with respect to survival and reproductive success within a modelled time period and, for better comparability, the PM results presented in Figure 61 also assume that the population of males has the same genetic makeup as that of females, and that the development period between reproduction and maturity of new offspring is one cycle.

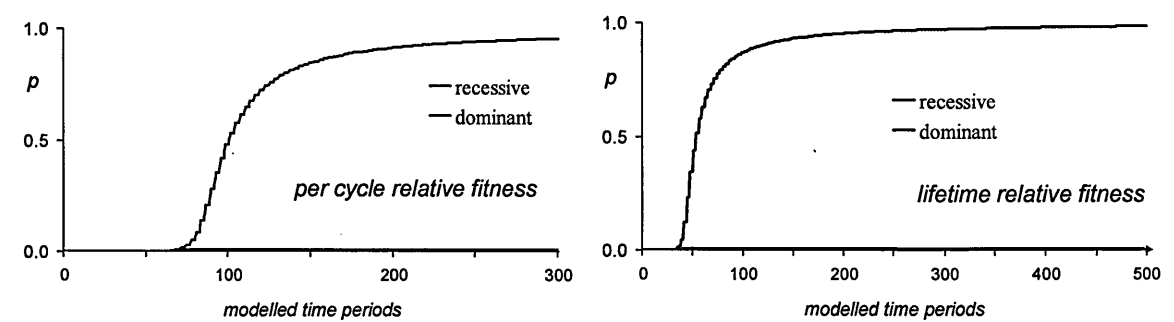
**Figure 61 Spread of resistance alleles over time, comparison of RM and PM results**  
 Spread of resistance alleles calculated using the PM model for a CIKI insecticide, assuming 1 cycle between egg production and resultant adults joining the population and that the male population has the same genotype as the female population (panel A), and using the RM model with the fitness benefit of resistance, set to 5.5 (panel B) and 2 (panel C). The plots in each panel show results assuming recessive and dominant resistance. All calculations assume  $p_0 = 0.00000001$ .



If the PM inputs are further adjusted to assume no adult survival between modelled time periods, the results become qualitatively much more like those from the RM, whether we maintain the same overall reproductive success per modelled time period, or use per lifetime reproductive figures in each modelled time period, as can be seen in Figure 62. The recessive allele does not spread in these examples within the 5,000 periods modelled by the PM.

**Figure 62 Spread of resistance alleles over time using the PM model with no adult survival between modelled periods**

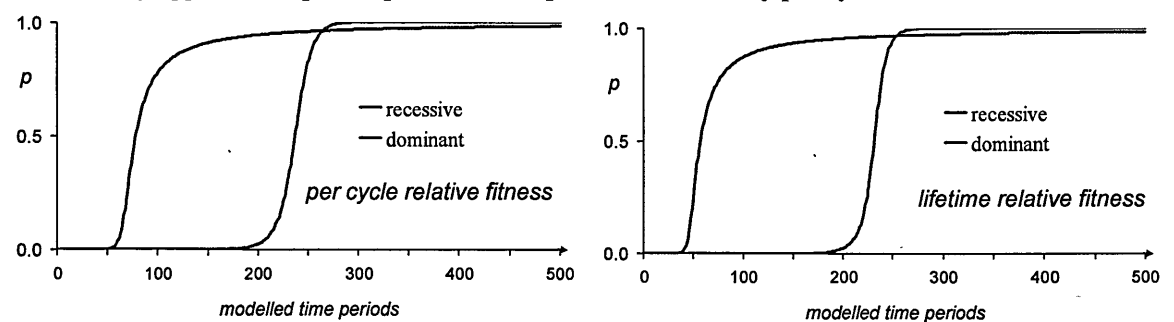
Using relative reproductive success values in each modelled time period equal to those per cycle (left hand panel), resistants produce 3× as many eggs as susceptibles, or per lifetime (right hand panel), resistants produce 6.5× as many eggs as susceptibles



Allowing new adults to survive and breed in a second time period is the simplest possible way to include overlap of part of the adult population between modelled time periods in the PM. We use per cycle resistant (65%) and susceptible (22%) survival probabilities between cycles 1 and 2 (Table 8), whilst either maintaining the per cycle reproductive value, or adjusting values to maintain the average per lifetime reproductive value across two cycles, and either assumption produces results much more consistent with the original PM results than with the RM results (Figure 63).

**Figure 63 Spread of resistance alleles over time using the PM model with 1 period survival of new adults between modelled periods**

Using relative reproductive success values in each modelled time period equal to that per cycle (left hand panel), resistants produce 3× as many eggs as susceptibles, or giving the original per lifetime value taking account of survival and reproduction in 2 periods (right hand panel), resistants produce 6.5× as many eggs as susceptibles per lifetime, equal to 4.8× as many per cycle



This suggests that population overlap may be a key assumption driving the difference between RM and PM results in cases with initially rare, recessive resistance alleles. To explore this further, eliminating any of the other possible differences between the PM and the RM, we add a generation overlap calculation to the RM model.

### 6.2.1 Overlap Model

The ‘replacement model’ (RM) calculates sequential values for  $p_i$  as

$$p_{i+1} = \frac{p_i^2 (1+s) + p_i q_i (1+hs)}{1+s(p_i^2 + 2hp_i q_i)}$$

Variables in the model are defined as follows;  $p_i$  is the proportion of resistance alleles in the population at start of time period  $i$ ,  $s$  is the fitness benefit of the resistant phenotype associated with a homozygous resistant genotype, and  $h$  is the proportion of that fitness benefit applicable to heterozygotes. If no direct fecundity effects of resistance are assumed, relative fitness is the same as the relative probabilities of resistant and susceptible phenotypes surviving through one modelled time period and hence forming part of the adult population at the start of the next period.

This is a representation of an iterative calculation based on proportions in the population of the three genotypes, homozygous susceptible, heterozygous and homozygous resistant. With the proportion of susceptible homozygotes in the population in modelled period  $i$  represented by  $a_i$ , of heterozygotes by  $b_i$  and resistant homozygotes by  $c_i$ , the proportions of each genotype in the population are calculated under the assumptions of the RM as follows (using superscript  $R$  to indicate values generated using the RM);

$$c_{i+1}^R = \frac{(c_i^R + 0.5b_i^R)^2 (1+s)}{\left(1 + (c_i^R + 0.5b_i^R)^2 s + 2(c_i^R + 0.5b_i^R)(1 - (c_i^R + 0.5b_i^R))hs\right)}$$

$$b_{i+1}^R = \frac{2(c_i^R + 0.5b_i^R)(1 - (c_i^R + 0.5b_i^R))(1+hs)}{\left(1 + (c_i^R + 0.5b_i^R)^2 s + 2(c_i^R + 0.5b_i^R)(1 - (c_i^R + 0.5b_i^R))hs\right)}$$

$$a_{i+1}^R = 1 - b_{i+1}^R - c_{i+1}^R$$

$$p_{i+1}^R = 0.5b_{i+1}^R + c_{i+1}^R$$

Seeking to avoid introducing ambiguity through unnecessary complexity, we use a simple development of the RM to explore the effects of including overlap of generations.

We assume that a proportion,  $m$ , ( $0 \leq m \leq 1$ ), of the population in each period comprises new offspring, and  $(1 - m)$  is the proportion of the population in each period comprised of surviving adults from previous periods, which we characterise as ‘overlap’. When  $m = 1$ , the revised model simplifies to the original RM model.

If we maintain the RM assumption that reproduction is periodic rather than continuous, and assume that modelled time periods equate to one reproductive cycle, the RM and the overlap model (hereafter “OM”) can use the same survival differential between resistant and susceptible phenotypes per reproductive period. The RM calculations ignore adults which survive from one period to the next, considering only the genotypes and resistance phenotypes of the offspring generated in each period. On this basis, the two models as formulated can have the same values for  $s$ , the fitness benefit accruing to the phenotype of a homozygous resistant genotype during a single modelled period, and results can be compared directly. Since for the RM, the fitness benefit of resistance can be equated to the differential survival of resistants through a period, from ‘birth’ to reproduction, we also use  $s$  as the value for differential survival from one modelled period to the next.

For the OM, genotype and allele proportions are calculated as follows, (using superscript  $V$  to indicate values generated using the OM);

$$c_{i+1}^V = m \frac{(c_i^V + 0.5b_i^V)^2 (1+s)}{(1 + (c_i^V + 0.5b_i^V)^2 s + 2(c_i^V + 0.5b_i^V)(1 - (c_i^V + 0.5b_i^V))hs)} + (1-m) \frac{c_i^V (1+s)}{(1 + c_i^V s + b_i^V hs)}$$

$$b_{i+1}^V = m \frac{2(c_i^V + 0.5b_i^V)(1 - (c_i^V + 0.5b_i^V))(1+hs)}{(1 + (c_i^V + 0.5b_i^V)^2 s + 2(c_i^V + 0.5b_i^V)(1 - (c_i^V + 0.5b_i^V))hs)} + (1-m) \frac{b_i^V (1+hs)}{(1 + c_i^V s + b_i^V hs)}$$

$$a_{i+1}^V = 1 - b_{i+1}^V - c_{i+1}^V$$

$$p_{i+1}^V = 0.5b_{i+1}^V + c_{i+1}^V$$

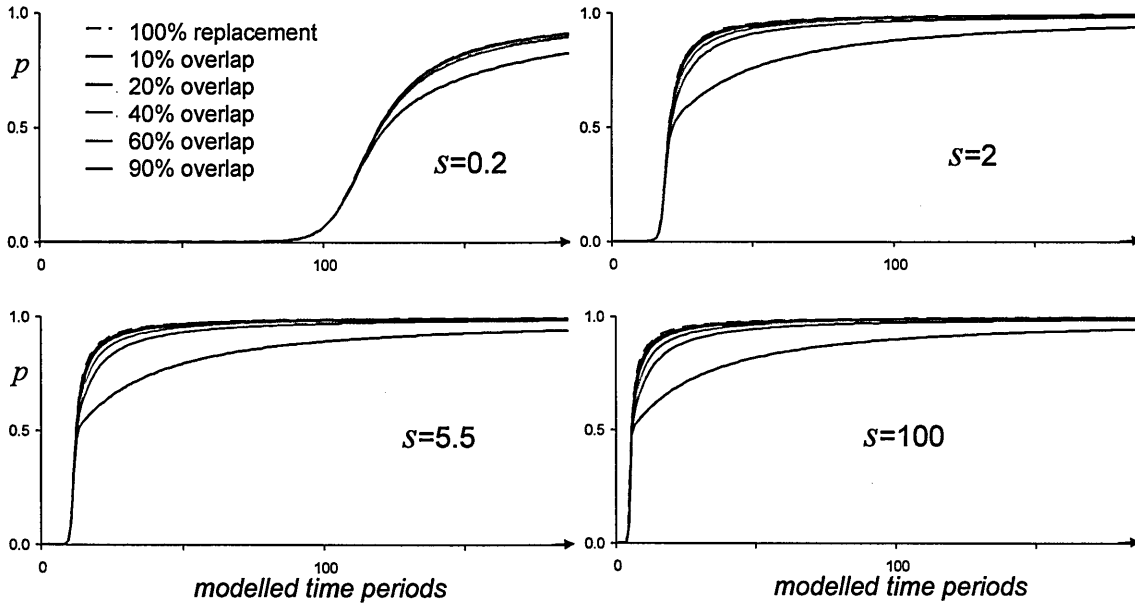
Graphical comparison of results calculated with the RM and OM, indicates (Figure 64) that



qualitatively similar results are calculated by the two models when heterozygotes are assumed to be largely or wholly resistant (resistance is dominant). In fact, if the proportion of resistant phenotypes is plotted rather than the proportion of resistant alleles, the OM and RM results with dominant resistance are effectively identical.

**Figure 64 Spread of dominant resistance allele calculated using the RM model and the OM model with a range of fitness benefits for resistance.**

Panels present results for values of  $s=0.2, 2, 5.5$  and  $100$ . Plots are for spread of dominant resistance alleles calculated using the RM, and using the OM assuming 10%, 20%, 40%, 60% and 90% overlap. For all results,  $p_0=0.000000001$



We therefore focus on comparison of the models when resistance is assumed to be recessive, ( $h = 0$ ). Under this assumption heterozygotes have no fitness or survival benefits, hence, spread of the resistance allele will depend on values of  $c$ , the proportion of resistant homozygotes.

Given  $h = 0$  we can restate the models as follows;

$$c_{i+1}^R = \frac{(c_i^R + 0.5b_i^R)^2 (1 + s)}{1 + (c_i^R + 0.5b_i^R)^2 s}$$

$$b_{i+1}^R = \frac{2(c_i^R + 0.5b_i^R)(1 - (c_i^R + 0.5b_i^R))}{1 + (c_i^R + 0.5b_i^R)^2 s}$$

$$p_{i+1}^R = \frac{(c_i^R + 0.5b_i^R)(c_i^R s + 0.5b_i^R s + 1)}{1 + (c_i^R + 0.5b_i^R)^2 s}$$

and

$$c_{i+1}^V = m \frac{(c_i^V + 0.5b_i^V)^2 (1+s)}{(1 + (c_i^V + 0.5b_i^V)^2 s)} + (1-m) \frac{c_i^V (1+s)}{(1 + c_i^V s)}$$

$$b_{i+1}^V = m \frac{2(c_i^V + 0.5b_i^V)(1 - (c_i^V + 0.5b_i^V))}{1 + (c_i^V + 0.5b_i^V)^2 s} + (1-m) \frac{b_i^V}{(1 + c_i^V s)}$$

$$p_{i+1}^V = m \frac{(c_i^V + 0.5b_i^V)(1 + c_i^V s + 0.5b_i^V s)}{1 + (c_i^V + 0.5b_i^V)^2 s} + (1-m) \frac{0.5b_i^V + c_i^V (1+s)}{1 + c_i^V s}$$

For any given non-zero initial values of  $b_i$  and  $c_i$  therefore, the rate of spread of the resistance allele calculated using the OM model  $\frac{p_{i+1}^V}{p_i^V}$  will be faster than that calculated

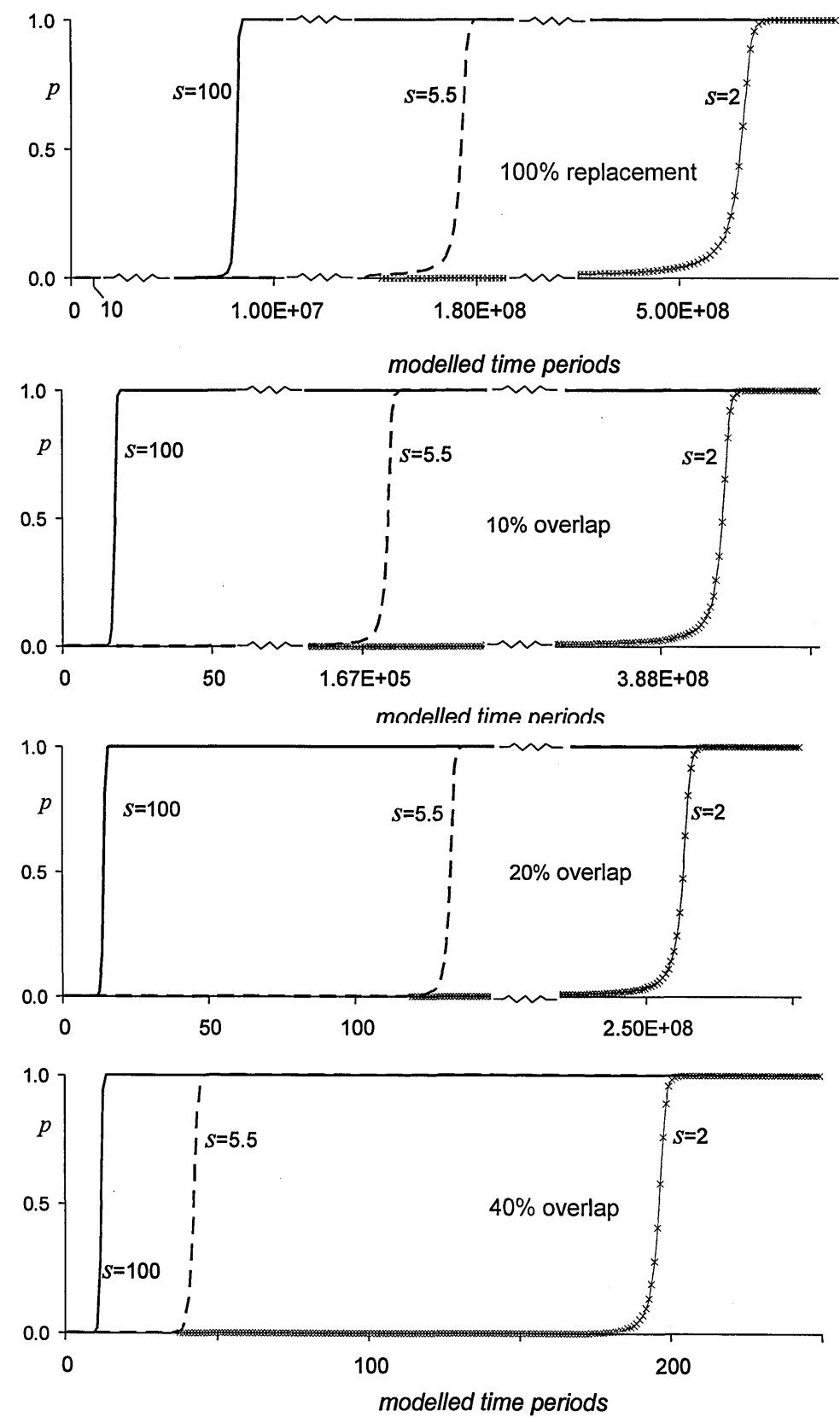
using the RM model,  $\frac{p_{i+1}^R}{p_i^R}$ , if  $c_i^V > \frac{(0.5b_i^V)^2}{(1 - 0.5b_i^V)}$  (note this is a sufficient but not necessary

condition).

Initial values of  $c_0$  may, however be expected to be very low or zero, in which case the OM will give a lower value for  $c_1^V$  than the RM's value,  $c_1^R$ . We can nonetheless show numerically that the RM understates the speed of spread of rare, recessive resistance alleles for overlapping generations even when a resistance allele is initially present only in heterozygotes and hence  $c_0=0$ .

**Figure 65 The spread of resistance alleles assuming a range of values for generation overlap,  $p_0=0.000000001$**

Spread of resistance alleles calculated with RM and OM models assuming fitness benefits of resistance of 100, 5.5 and 2. Panels are for (top to bottom) RM, OM assuming that 10%, 20% or 40% of the population in each time period comprises adult survivors from the preceding time period. In all cases resistance alleles are initially assumed to be present in heterozygotes only, and to comprise 1E-9 of the population. Note that x-axis values differ between panels.

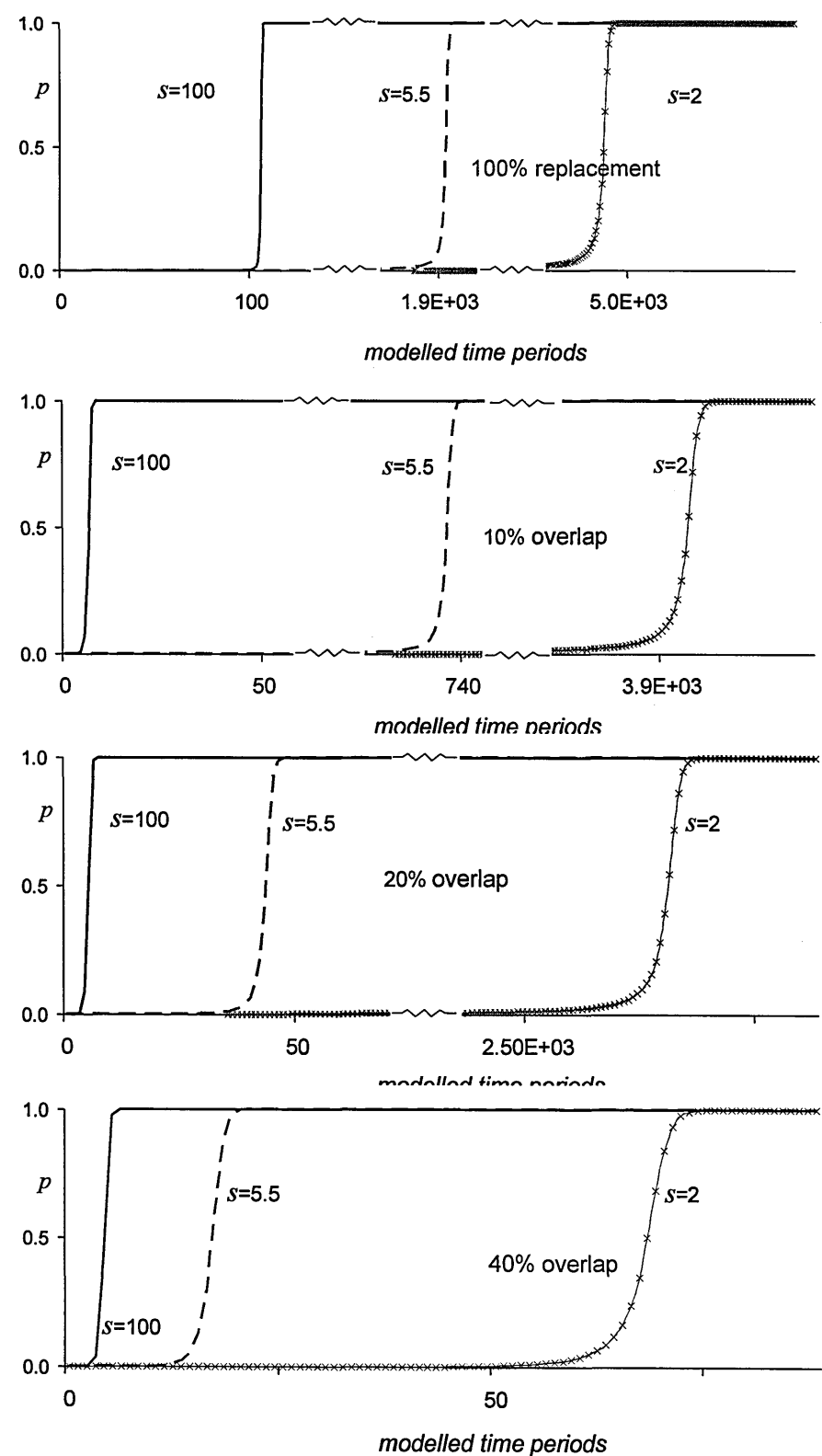


It can be seen from the results shown in Figure 65 that for a given fitness benefit of resistance,  $s$ , the calculated value of  $p$  increases more slowly using the RM than using the OM with any of the tested values for generation overlap,  $(1-m)$ , between model periods. It can further be seen from Figure 65 that  $p$  increases more rapidly with higher values for overlap, and that the effect of overlap is greater with higher values of  $s$ , the relative fitness benefit of resistance.

If the initial proportion of resistance alleles  $p_0$ , is increased, the times to spread of resistance are predictably compressed, as the long, differential periods of slow initial spread are removed, Figure 66. As can be seen from comparison with Figure 65, the effect of overlap is greater with a smaller initial value of  $p_0$ . For example, for  $s=5.5$ , with  $p_0=10^{-9}$ , modelling a 20% overlap between generations reduces the time to spread of resistance from approximately  $2 \times 10^8$  to 100, a factor of about  $2 \times 10^6$ , the equivalent reduction with  $p_0=10^{-4}$  is from 2,000 to 40, a factor of approximately 50.

**Figure 66 The spread of resistance alleles assuming a range of values for generation overlap, with  $p_0=0.0001$**

Spread of resistance alleles calculated with RM and OM models assuming fitness benefits of resistance of 100, 5.5 and 2. Panels are for (top to bottom) RM, OM assuming that 10%, 20% or 40% of the population in each time period comprises adult survivors from the preceding time period. Note that x-axis values differ between panels.



### 6.3 Discussion

Our results show that generation overlap is one of the key determinants of the speed of spread of resistance for initially rare recessive resistance alleles. In all cases resistance is predicted to spread more rapidly when population overlap is assumed. The practical importance of this effect is at least partly determined by context rather than just by magnitude. For example, for a 5.5 fitness benefit of resistance, with  $p_0=10^{-9}$ , modelling a 10% overlap indicates a time to resistance approximately 100 fold less than that indicated by the RM. Depending on the actual period of time represented by the modelled periods, however, the difference between  $1.8 \times 10^8$  and  $1.7 \times 10^5$  may be immaterial, both timescales effectively representing 'never'. The same comparison assuming  $p_0=0.0001$ , calculates resistance spreading in around 1,900 periods using the RM, reducing to less than 740 periods when 10% overlap is modelled. If modelled periods equate to a year, this difference is also of little practical importance, but if the modelled time period is some smaller unit, halving the expected time to spread of resistance may be a very important issue. The difference in expected time to spread of resistance for a fitness benefit of 100 with  $p_0=10^{-9}$ , from around 10 million periods, modelled assuming no overlap of generations, to around 10, modelled with just 10% overlap, would almost certainly be seen as having very serious practical implications in any context, however.

In assessing the significance of these results it is helpful to consider that  $s=100$  is the order of magnitude of fitness benefits of resistance for pests feeding on crops genetically modified to express Bt toxins [94,101], our FCM model (Table 12) calculates the per cycle fitness benefit of resistance to a conventional insecticide as  $s=2$ , and of resistance to a 4-cycle ADI as  $s=0.2$ . Also, based on an assumption that a constant number of newly mature offspring join the population in each modelled feeding cycle, the PM model calculates values for  $m$  of approximately 78% (i.e. 22% overlap) for a susceptible population, increasing as the proportion of resistant phenotypes grows, up to 64% (36% overlap) for a

wholly resistant population. The values tested in our analysis are therefore within a biologically meaningful range.

In some circumstances the dominance of an allele is context-sensitive. For example, a resistance allele giving heterozygotes resistance to a given insecticide only for concentrations below a given threshold, would produce resistant heterozygotes in a context where the insecticide was present in low concentrations, and susceptible heterozygotes in a context where the insecticide was present in high concentrations[100]. Based on this it has been suggested that some resistance management strategies would actually result in an increase in the speed of spread of resistance if they generated a switch from functional recessivity to functional dominance for resistance alleles [102]. It is clear that such concerns are in part predicated on the assumption that rare recessive resistance alleles will, in practical terms, not spread. This assumption rests on the use of models equivalent to the RM, and whilst there is no reason to question its validity for life histories complying with the assumption of discrete non-overlapping generations, our work suggests it should be questioned when this is not the case.

The results we generate using the OM model all assume a constant value for the proportion of the population in each model period which is comprised of new offspring rather than surviving adults ( $m$ ). Many factors can affect this value, including numbers of offspring produced per breeding adult, juvenile and adult survival, any adult or juvenile density dependence effects, *et cetera*. It cannot be derived directly from the parameters of the OM model without making additional explicit assumptions, such as the assumption of constant recruitment used for the analyses in chapters 3 and 4. In most circumstances, however, one might expect that changes to average adult survival probabilities, as arise when resistant phenotypes replace susceptibles in a population, will produce changes in the relative proportions of new offspring and surviving adults at the start of each modelled period. Since the value of  $m$  directly affects the speed of spread of resistance (Figure 65 and Figure 66) variations in this value as resistance spreads might alter the profile of  $p$  over time, but

for the purposes of comparison with results from RM, this should not affect our conclusions since the value of  $m$  during the crucial early periods, when spread of the allele accelerates, will be relatively stable, as susceptibles consistently form the vast majority of the population during this period.

## **6.4 Conclusions**

It can be seen from our numeric analysis that the assumption of discrete, non-overlapping generations is key to the applicability of simple, single-allele, population genetics models when evaluating the spread of rare, recessive resistance alleles. Applying such models to organisms with life histories which violate this assumption is likely to generate highly misleading results, particularly when the fitness benefit of resistance is high and/or the proportion of the breeding population comprised of adult survivors rather than newly mature offspring is high. In such instances, the conventional wisdom, that rare, recessive resistance alleles will never spread, is wrong.



## 7 Chapter 7 General Discussion and Conclusions

In the preceding chapters we have explored a number of very specific questions under the general title "Mathematical modelling of the effects of health interventions on the evolution of life history in disease-causing organisms" and drawn particular conclusions with respect to each. Are any general conclusions possible for this group of topics? One consistent theme, perhaps, is the idea that detail matters. For worm evolution under health interventions, the precise details of worm mortalities will determine the direction of selection. For LLA insecticides, it is the detail of mortality schedules which opens the opportunity to combine transmission reduction with minimal selection for resistance, and for the standard popgen model, details of population structure prove critical to its results. Many biological parameter values are currently unknown and may be impossible to determine, so if detail matters, does this preclude any meaningful modelling of biological systems? Does all the work detailed in this text simply serve to highlight the futility of such undertakings? To paraphrase the Guy Browning quote heading chapter 1, how do we model soup?

If they collectively serve to pose this question, do the chapters above also offer a collective answer? On reflection, we would suggest that they do, and that, paradoxically, they show that the way to deal with complexity when detail matters, is to simplify. In chapter 2 we generate a model constructed around mortality functions which are currently unknowable. We cannot therefore populate our models with functions and parameters capturing the reality of specific parasite mortality schedules, with or without interventions, and we cannot realistically attempt to answer the question "what will happen?". However, if we define the simplest result which can answer our original question, we merely need to know in which direction selection may push, and we need only consider the fact that interventions will increase worm mortality, not the details of the resultant mortality

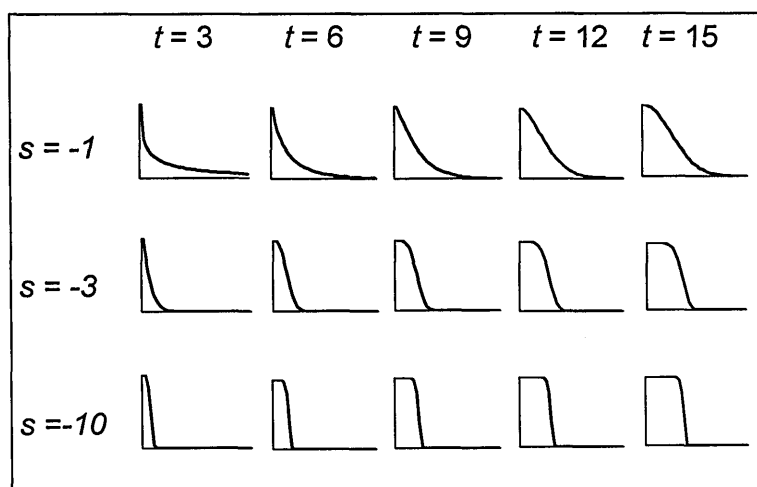
schedules. The question is “what may happen?” and to answer that question we don’t need to know any mortality functions or parameter values.

In chapter 3 we need a quantitative answer to whether LLAs can provide a combination of transmission control and resistance management. Although indicative values are available for some of the relevant life-history parameters, we lack not only well-defined parameter values, but also knowledge of the existence and/or significance of many aspects of the life-histories of mosquitoes, *Plasmodium*, and humans which might influence the precise number of infections transmitted by a mosquito, or the precise number of viable offspring it may produce. Here, again, we can simplify our question; rather than “what will happen if we change this” we ask “how much better or worse will things be if we change this?”. By formulating our results in comparative terms, we greatly reduce the significance of the unknown and unknowable aspects of the life-histories we are modelling, and remove the necessity of attempting to capture them in the model. Our sensitivity analysis confirms that, whilst the absolute calculated values for eggs laid and infectious bites given are very sensitive to some parameters, the comparative results remain robust to variations in parameters not directly related to the interventions we are assessing.

Simplicity also contributes to our evaluation of optimal virulence characteristics for fungal biopesticides. With an infinite range of possible survival curves, how do we produce generalisable results which can guide a practical process of strain selection? Elegant mathematical functions which replicate any curve are available, but the parameters which define such curves do not translate easily into biologically meaningful terms (Figure 67). Review of the basic biology leading to consideration of fungal entomopathogens as potential LLAs, the characteristic initial period of development prior to the onset of fungus-induced mortality, gave rise to the ‘simple virulence’ definition used in chapter 4 which proved intuitive and easy to work with for this purpose (although tragically inelegant).

### Figure 67 Sample curves generated using the Weibull distribution

The curves generated by the Weibull distribution are defined by two parameters 's' and 't', which provide an elegant representation of almost any conceivable shape of survival curve, but do not give an obvious intuitive link to the biological parameters of interest.



For the analysis based on experimental results in chapter 5, the whole process of deriving values useable in the model was one of simplification, supported by sensitivity analysis, to check that we hadn't lost any crucial detail in the simplifying process.

In chapter 6, which is most explicitly about the importance of detail, it is nonetheless only by simplifying that we reached any meaningful conclusions. There are many differences between our age-structured vector PM model and the RM model. Only by eliminating as much of the complexity as possible could we establish that population overlap is a key driver of the dramatic difference in speed of spread of resistance alleles calculated by the two models.

Although we were able to address the specific questions which defined each of the above topics, and to reach conclusions based on our results, a number of other questions, beyond the scope of this text, are worth commenting on.

In section 3.6.6 we compare results calculated using two different population assumptions, either constant numbers of new adults joining the population in each period, or constant adult population size. It would be interesting to develop a new version of the model which could incorporate juvenile and adult density dependence, and ratios of offspring to breeding adults, so that the effects of various alternative assumptions could be assessed,

such as seasonal variation in population size, and the effect of offspring numbers which change in line with the changing size of the adult population.

A potential mode of action for an LLA which cannot be evaluated by our model without further development is bioaccumulation, whereby repeated doses of a pesticide accumulate to reach a fatal dose. Assuming that coverage is less than 100% and/or the probability of feeding on a non-human host  $>0\%$ , this would not be directly equivalent to any of the LLA types evaluated so far, and it may be interesting to explore the possibilities, either for novel chemistry, or as a novel mode of delivery for existing chemicals.

Although our numeric analysis justifies our conclusions in chapter 6, that population overlap is a key driver of the spread of rare recessive alleles, it would be pleasing to extend our mathematical result to illuminate why this appears to be true in all circumstance, not just for the initial conditions already identified.

Chapters 3, 4, and 5 all consider the use of novel insecticides within the existing framework of delivery in an indoor setting via IRS or ITNS. We explicitly define behavioural avoidance as a sub-category of resistance, and do not consider it as a separate issue. In terms of resistance management this seems a reasonable assumption. However, the evolution of outdoor biting behaviour [103,104] poses many questions about the sustainability of transmission control based on indoor delivery methods. No data is currently available about the fitness costs associated with changes to mosquito feeding patterns. Analysis of the effects of deflection by bed nets have focussed on the direct effect on disease control, usually by contrasting the effect of deflection with that of mosquito death in terms of preventing infectious bites, but have not attempted to quantify the fitness costs to the mosquito. To the best of our knowledge field data is not available regarding the fitness costs to the mosquito of deflection to alternative indoor hosts or to outdoor feeding. Since the widespread use of indoor insecticides and bed nets has not been seen to produce a rapid switch to outdoor biting, we must assume that there are either practical constraints

which prevent this, or fitness costs which outweigh the costs of encountering insecticides during indoor feeding. Most *Anopheles* species preferentially feed indoors at night, biting sleeping hosts. There are many possible causes for this behaviour, for example, it may be safer to feed on humans when they are asleep, therefore vectors feed at night and indoors, because that's when and where sleeping people are found. Alternatively, vectors may experience lower mortality by being active at night, and at night people are asleep and indoors, so that is where mosquitoes feed. If the former is the case, people choosing to sleep outdoors at any time of day would offer the best alternative hosts to people sleeping in buildings protected with IRS or ITNs. If the latter applies, then people outside at night would offer the most likely alternative hosts for mosquitoes avoiding indoor insecticides. Different fitness costs would be incurred in each case by mosquitoes switching strategies and without a proper understanding of such costs, intelligent management of this form of resistance cannot be confidently developed in theory, much less applied in practice.

Whether as a problem to be resolved, or a process to be exploited, evolution must be taken into account when we make choices and design interventions. Ferguson et al [105] consider the possibility that public health interventions have already driven the evolution of malaria vectors on a grand scale, changing mosquito life-histories and consequently selecting for increased refractoriness to *Plasmodium* throughout Africa. In chapter 2 we discuss the idea of working with evolution, adjusting interventions not merely to avoid unwanted evolution, but to target desirable outcomes, Ferguson et al discuss this idea with respect to malaria vectors. At a time when it is still difficult to engage health professionals with the reality of resistance evolution as a pressing and immediate problem, it seems unlikely that they will be ready to respond to strategies designed to enlist evolution to act on their behalf. Can we stop seeing evolution generated by health interventions as inevitably threatening, and instead try to understand and direct it intelligently in directions which help us? Let's hope so.

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**Several parts of this thesis  
gave rise to or contributed to  
the following papers**

# How will public and animal health interventions drive life-history evolution in parasitic nematodes?

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## SUMMARY

Infection caused by parasitic nematodes of humans and livestock can have significant health and economic costs. Treatments aimed at alleviating these costs, such as chemotherapy and vaccination, alter parasite survival and reproduction, the main selective pressures shaping life-history traits such as age to maturity, size and fecundity. Most authors have argued that the life-history evolution prompted by animal and public health programmes would be clinically beneficial, generating smaller, less fecund worms, and several mathematical models support this view. However, using mathematical models of long-lasting interventions, such as vaccination, and regularly repeated short interventions, such as drenching, we show here that the expected outcome actually depends on how mortality rates vary as a function of worm size and developmental status. Interventions which change mortality functions can exert selection pressure to either shorten or extend the time to maturity, and thus increase or decrease worm fecundity and size. The evolutionary trajectory depends critically on the details of the mortality functions with and without the intervention. Earlier optimism that health interventions would always prompt the evolution of smaller, less fecund and hence clinically less damaging worms is premature.

**Key words:** mortality rates, health interventions, maturation time, vaccination, chemotherapy, anthelmintic.

## INTRODUCTION

Infections by parasitic nematodes have a large impact on the health of humans and domestic livestock. Two key life-history traits, fecundity and body size, are important determinants of nematode infectiousness and host damage (Skorping, Read and Keymer, 1991; Stear, Strain and Bishop, 1999). Both are a consequence of the age at which nematodes mature. All other things being equal, it takes longer to get bigger, and nematode growth stops or rapidly declines after reproduction begins. Moreover, bigger worms can produce more eggs (Skorping *et al.* 1991; Morand, 1996; Gemmill, Skorping and Read, 1999; Leignel and Cabaret, 2001; Sorci *et al.* 2003). Consequently, age at maturity must be subject to intense natural selection. Here we ask how health interventions, such as widespread vaccination and chemotherapy, might alter nematode life history evolution. Most previous work has shown that smaller, less fecund worms are the likely outcome (Medley, 1994; Poulin, 1998; but see Skorping and Read, 1998; Gemmill *et al.* 1999). In this paper we show that a variety of evolutionary outcomes is

possible, including the evolution of larger and hence more fecund and damaging worms.

Previous theoretical work on the evolution of parasitic nematode life-histories has followed standard life history theory (Roff, 1992; Stearns, 1992) and assumed that mortality schedules are the major determinants of selection (Skorping *et al.* 1991; Morand and Sorci, 1998; Gemmill *et al.* 1999; Morand and Poulin, 2000; Sorci *et al.* 2003). Where chances of survival are high, nematodes should delay maturity to gain the fecundity benefits of large size. However, when chances of survival are low, worms should mature early in order to achieve some reproduction before death, even if this means they mature at small size and hence have low fecundity. Thus, where daily survival rates are high, one might expect a life history like that of *Ascaris lumbricoides*, for example, which reaches up to 30 cm in length and produces 25 million eggs over a lifetime. In contrast, where chances of survival are low, natural selection should favour a life-history like that of the pin worm, *Enterobius vermicularis*, which has a maximum length of 1 cm and produces no more than 20 000 eggs. A formal model of this idea, together with experimental data on survival rates, explains about 50 percent of the cross-species variation in age to maturity of parasitic nematodes of mammals (Gemmill *et al.* 1999).

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The aim of animal and human health programmes like chemotherapy and vaccination is to reduce worm survival. Thus, nematode life-histories could evolve in response to public and animal health programmes (Medley, 1994; Read and Skorping, 1995; Poulin, 1998; Skorping and Read, 1998; Leignel and Cabaret, 2001). This evolution may in principle occur in parallel with, or instead of, the evolution of drug or vaccine resistance. There is no direct evidence yet of such evolution, but it has not to our knowledge been looked for (for indirect evidence, see Leignel and Cabaret, 2001). In other contexts, where it has been looked for, life-history evolution in response to anthropogenic alterations in mortality schedules has been demonstrated. For instance, size-selective harvesting of populations of Atlantic silverside (*Menidia menidia*) changed size-dependent mortality schedules, and produced rapid evolution of slow growing, smaller fish in large-harvested populations and fast-growing, larger fish in small-harvested populations (Conover *et al.* 2005).

Most previous theoretical work on the evolution of nematode age in response to medical and veterinary intervention has suggested that the resulting life-history evolution would be beneficial from a disease control standpoint. The argument is that intervention-induced increases in mortality will mean that natural selection will always favour earlier maturation and thus result in smaller and less fecund worms (Medley, 1994; Poulin, 1998; Gemmill *et al.* 1999). However, existing formal models of this make fairly restrictive assumptions about the nature of nematode mortality patterns, in particular assuming that mortality rates are unaffected by age at maturity. Here we formally analyse earlier verbal suggestions (Read and Skorping, 1995; Skorping and Read, 1998; Gemmill *et al.* 1999) that some types of stage- or size-specific mortality might generate clinically-detrimental life history evolution.

It seems highly likely that mortality rates will vary with worm size. Larger nematodes presumably provide more stimulus to the immune system, all else being equal, because they will secrete more antigens and have a larger surface area, and may do more damage. Alternatively, smaller nematodes may be more vulnerable to immune attack if they are less able to withstand damage from a given number of effector molecules. The host immune response can also alter worm fecundity directly and indirectly via its effects on worm size (Wilkes *et al.* 2004; Viney, Steer and Wilkes, 2006). Moreover, immunity can differentially affect the survival of different developmental stages of parasites. For example, in *Strongyloides ratti* different mortality rates were observed for larval and adult stages which are in different host tissues (Bell, Adams and Gerb, 1981). Here we consider the effects of chemotherapy and vaccination allowing for these sort of more complex mortality schedules. We also consider the effects

both of changes in mortality schedules which might be continuous (e.g. vaccination or, in the case of farm animals, artificially-selected resistant hosts) or those which would be pulsed (e.g. many chemotherapeutic regimes used in an agricultural context). We show that optimism emerging from previous models may be misplaced: in some circumstances, animal and public health interventions may select for increased time to maturity, which would result in larger and more fecund worms.

## MODELS

Here we consider the size-independent mortality model (henceforward “SIM” model) developed by Gemmill *et al.* (1999), and introduce our new model, which incorporates size-dependent mortality (henceforward “SDM” model). We then use these models to study the effect of public and animal health interventions on worm life-history evolution. In a subsequent section, we develop a model to study the effect of size-dependent mortality when there are pulsed interventions like regular drenching of farm animals with anthelmintics (henceforward “SDMP” model). All models assume that worm births are steady over time and the population is in equilibrium, hence lifetime reproductive success (measured as lifetime egg production) is an appropriate measure of fitness. Anderson and May (1985) provide evidence supporting this assumption. Analysis of the epidemic situation, where other fitness measures are more appropriate, is beyond the scope of this paper.

Throughout, symbols are as given in Table 1, and all mortality rates are instantaneous mortality rates – the probability of death at any particular point in time.

### Size independent mortality model

The assumptions of this model are as follows (Gemmill *et al.* 1999): (1). Worms grow throughout development, but growth ceases at maturity. (2). Per unit time fecundity increases with worm size and hence with maturation time  $\alpha$ , according to the relationship fecundity =  $c\alpha^\beta$ . (3). Within the host, parasites experience a constant juvenile mortality rate,  $M_j$ , until maturation. (4). After the onset of reproduction, parasites experience a constant adult mortality rate,  $M_a$ .

The probability of survival to maturation at time  $\alpha$  is derived by treating the occurrence of death as a random variable with distribution Poisson( $\lambda$ ) where  $\lambda$  is the mortality rate,  $M_j$ . Thus, the average lifetime fecundity for individuals maturing at  $\alpha$  is given by

$$\omega = c\alpha^\beta e^{-M_j\alpha} \frac{1}{M_a} \quad (1)$$

Table 1. Variables and Parameters for SIM, SDM and SDMP models. Note all ages are measured from first infection of the mammalian host

$\alpha$	Age at maturity
$\omega(\alpha)$	Fitness of worms maturing at $\alpha$
$c$	Constant relating age at maturity to worm fecundity
$\beta$	Exponent of allometric relationship relating age at maturity to fecundity
$M_j$	Within-host mortality rate for juvenile parasites
$M_a$	Within-host mortality rate for adult parasites
$m(z)$	Mortality rate experienced by juvenile parasites at age $z$
$d(\alpha)$	Mortality rate experienced by adult parasites which matured at age $\alpha$
$\omega_h(\alpha)$	Fitness of worms maturing at $\alpha$ in hosts experiencing a health intervention
$\beta_h$	Allometric exponent relating fecundity to age at maturity in hosts experiencing a health intervention acting to reduce rate of increase of fecundity with age
$m_h(z)$	Mortality rate experienced by juvenile parasites at age $z$ in hosts experiencing a health intervention acting to increase juvenile parasite mortality
$d_h(\alpha)$	Mortality rate experienced by adult parasites which matured at age $\alpha$ in hosts experiencing a health intervention acting to increase adult parasite mortality
$s_h(\alpha^*)$	Selection gradient at $\alpha^*$ under an intervention
$I$	Time interval between doses; ( $I > \alpha$ )
$H$	Proportion of hosts dosed during dosing events
$D_j$	Probability of juvenile parasites dying as a result of dosing event, if in dosed host
$D_m$	Probability of adult worms dying as a result of dosing event, if in dosed host
$t$	Time from start of interval between dosing events; ( $0 < t < I$ )
$\omega_p(\alpha)$	Overall average fitness of parasites maturing at age $\alpha$ under pulsed dosing

The model comprises three elements:  $c\alpha^\beta$ , the daily fecundity following maturity at  $\alpha$ ,  $e^{-M_j\alpha}$  the probability of survival to maturity with pre-patent period  $\alpha$ , and  $\frac{1}{M_a}$  the life expectancy post-maturity (assuming survival times are exponentially distributed).

The age at maturity favoured by natural selection,  $\alpha^*$ , corresponds to the maximum of  $\omega(\alpha)$ , at which the derivative  $\omega'(\alpha^*)=0$ , namely

$$\alpha^* = \frac{\beta}{M_j} \quad (2)$$

The same result can be derived from an explicitly epidemiological framework (Appendix A).

#### Size-dependent mortality model

We now extend the size-independent model (equation (1)) to include size-dependent mortality before and after maturation. In the next section, we use this framework to explore the effects of health interventions on optimum time to maturity.

To incorporate size-dependent mortality, we replace assumptions (3) and (4) above with the following: (5). Pre-maturity mortality rate is determined by size, and so changes during larval development. It is given by the function  $m(z)$ , where  $z$  is the time (age) from arrival in host. (6). Adult parasites experience constant mortality, determined by the size at which they matured, and given by the function  $d(\alpha)$ .

The size-dependent mortality model has a mortality rate which varies with time, and so the occurrence of death is a non-homogeneous Poisson process with distribution  $\text{Poisson}(m(z))$ . Thus, the

probability that death will not occur before age  $z$  is given by

$$1 - F(z) = e^{-\mu(z)}$$

where

$$\mu(z) = \int_0^z m(u) du \quad (z > 0)$$

Fitness is therefore given by

$$\omega(\alpha) = c\alpha^\beta e^{-\mu(\alpha)} \frac{1}{d(\alpha)} \quad (3)$$

which reduces to equation (1) for constant mortality rates  $m(z) = M_j$  and  $d(\alpha) = M_a$ .

The optimal value,  $\alpha^*$ , is again determined by the condition  $\omega'(\alpha^*)=0$ . Thus,

$$0 = \frac{\beta}{\alpha^*} - \frac{d'(\alpha^*)}{d(\alpha^*)} - m(\alpha^*) \quad (4)$$

with the additional requirement that, to ensure  $\omega(\alpha)$  is maximal at  $\alpha = \alpha^*$ , the second derivative must be negative.

As illustrated in Appendix B, multiple solutions may be possible for some combinations of mortality functions so that the theoretical global optimum may not always be the value selected for.

#### THE EVOLUTIONARY CONSEQUENCES OF PUBLIC AND ANIMAL HEALTH PROGRAMMES ON NEMATODE AGE AT MATURITY

Interventions like chemotherapy, vaccination and, in the case of animal diseases, enhanced host resistance

through selective breeding could affect many of the key functions and variables which shape the selection pressures on nematode age to maturity. For instance, enhanced host resistance or subcurative chemotherapy can reduce  $c$ , the absolute worm fecundity (e.g. Crook and Viney, 2005; Viney *et al.* 2006). It follows from equations (2) and (4) that this has no effect on the evolution of age to maturity whether or not there is size-dependent mortality. Similarly, if the adult mortality rate does not vary with age at maturity, then equation (4) reduces to equation (2) and changes to the absolute value of the adult mortality rate will also have no effect on selection for age at maturity. Otherwise, however, interventions which alter the juvenile mortality rate at a given age,  $m(z)$ , the adult mortality rate for worms maturing at a given age,  $d(\alpha)$  or the rate at which fecundity increases with age at maturity,  $\beta$ , will prompt evolutionary change in age to maturity. For instance, host immunity reduces the fecundity of *S. ratti*, by both reducing worm size and by reducing the fecundity of worms of a given size (Viney *et al.* 2006). It follows from (4) that where such effects occur, disease control interventions like mass vaccination which affect the immune environment experienced by a worm population will impose selection for altered age to maturity.

To understand the direction of this new selection, we consider two types of intervention. The first is where the entire natural life-span of the worms can be expected to fall within a period where the intervention is having an effect, as would be the case for immunisation or enhanced resistance by selective breeding; for simplicity we consider this under the general heading of 'sustained interventions'. The second is where the intervention acts as series of brief, regularly spaced, discrete events against the background of the underlying mortality rates, as occurs with chemotherapy in an agricultural context, where animals are routinely drenched at particular intervals. We refer to this as 'pulsed interventions'. These two situations need to be modelled in different ways, so we consider each in turn.

#### *The effects of sustained interventions on optimum time to maturity*

With size-dependent mortality, there is no generalised equation for  $\alpha^*$  analogous to equation (2). However, an indication of the immediate direction of selection on age to maturity under an intervention can be determined by the sign of the selection gradient, the derivative of the fitness function under the intervention, in the vicinity of the pre-intervention value of  $\alpha^*$ . This corresponds to the sign of  $s_h(\alpha^*)$  where

$$s_h(\alpha^*) = \frac{\beta_h}{\alpha^*} - \frac{d_h'(\alpha^*)}{d_h(\alpha^*)} - m_h(\alpha^*) \quad (5)$$

with one or more of  $\beta_h$ ,  $d_h(\alpha^*)$  and  $m_h(\alpha^*)$  affected by an intervention. When equation (5) is positive, the intervention is creating selection pressures that favour worms which grow for longer before reproduction; when equation (5) is negative, natural selection favours shorter maturation periods. Note that this selection gradient approach applies only in the immediate region of the pre-intervention  $\alpha^*$ . Where multiple solutions are possible (e.g. Appendix B), the overall direction of evolutionary change may be different.

Inspection of equations (5) and (4) reveals the following. All else being equal, a health intervention which changes the pre-maturity mortality function to  $m_h(z)$ , with greater mortality for a given size ( $m_h(z) > m(z)$ , for all relevant values of  $z$ ) will always favour reduced time to maturity. This is also true for size-independent mortality (equation (2); Gemmill *et al.* 1999). In both cases, this is because greater prematurational mortality selects for earlier reproduction, despite the fecundity costs, to ensure that worms survive to reproduce at all. Similarly, an intervention which changes the rate of increase of fecundity with size, so that worms are less fecund for a given size (i.e.  $\beta > \beta_h$  such that  $\beta_h < \beta$ ), will make  $s_h(\alpha^*) < 0$ , so that initial selection pressure will always favour a reduced time to maturity. This too is true for size independent mortality (equation (2); Gemmill *et al.* 1999), and is because the intervention is reducing the fecundity gains which accrue through delayed reproduction. Thus, interventions which increase juvenile mortality or decrease the rate of increase of fecundity with worm size will favour the evolution of an earlier age at maturity which will result in smaller and less fecund worms, whether or not mortality rates are size-dependent. These effects are illustrated in Fig. 1.

An intervention which affects mortality rates of mature worms has more complex effects on the optimal age to maturity. Inspection of equations (5) and (4) shows that the direction of selection under the intervention depends upon the difference between  $d'(\alpha^*)/d(\alpha^*)$  and  $d_h'(\alpha^*)/d_h(\alpha^*)$ , the proportionate rates of change in mortality with size before and after imposing the intervention. This difference depends in turn upon the detail of each function around  $\alpha^*$ . If the difference is positive, then the initial selection pressure will favour earlier maturing worms (Fig. 2a–c). If the difference is negative, as is always the case if the slope of  $d_h(\alpha)$  is less than or equal to that of  $d(\alpha)$ , then interventions to increase adult mortality will always favour worms which delay maturation (Fig. 2d–f and g–i). If age to maturity does not affect adult mortality, then the slopes of  $d(\alpha)$  and  $d_h(\alpha)$  will be zero, and the adult mortality rate imposes no selection on age to maturity (Gemmill *et al.* 1999).

To understand how changes in adult mortality can have these contrasting effects on age to maturity, it is



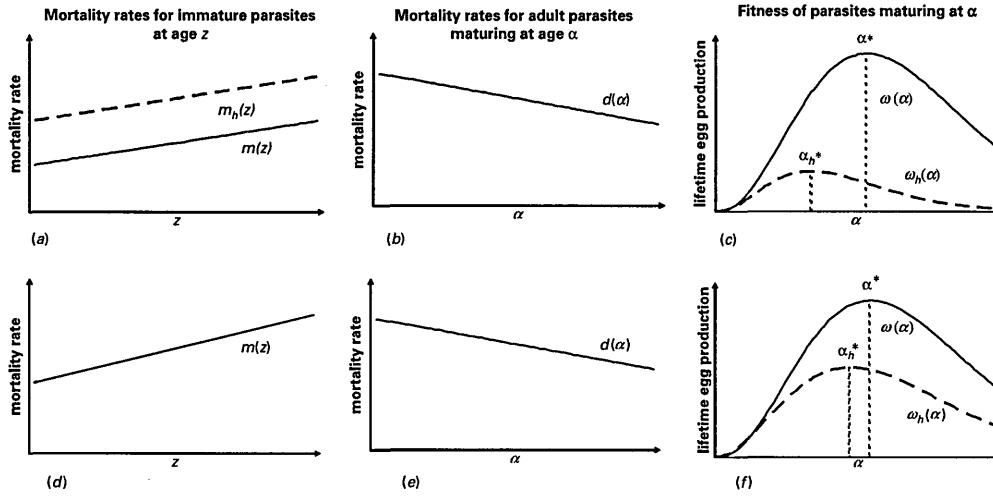


Fig. 1. Illustration of the effects of interventions which increase juvenile mortality or reduce fecundity. Panels (a) to (c) illustrate the effects on fitness of an intervention which increases the juvenile mortality rate from  $m(z)$  to  $m_h(z)$ , and panels (d) to (f) show the effect of an intervention which leaves the mortality rates unchanged but reduces the rate at which fecundity increases with age at maturity. In both cases the fitness function under the intervention reaches its maximum with a shorter time to maturity ( $\alpha_h^*$ ) than that without the intervention ( $\alpha^*$ ). Continuous lines show functions without the intervention, dashed lines with the intervention.

helpful to consider the situation before the intervention is imposed. At the optimum age to maturity,  $\alpha^*$ , there is the highest possible product from the three components of fitness: (i) chance of surviving to maturity, (ii) fecundity and (iii) duration of reproduction (adult life expectancy). By definition, worms maturing earlier or later than the optimum age will not have maximum fitness, so any associated improvement in one or more of the fitness components must be proportionately more than offset by a reduction in the other component(s). For example, worms beginning reproduction after the optimum age will have a relative fitness benefit from increased fecundity, but this benefit must be outweighed by a proportionately greater reduction in the product of their chance of surviving to maturity and their duration of reproduction.

Now consider an intervention which changes adult mortality rates and hence duration of reproduction, whilst the other two components of fitness remain unchanged. The proportionate rate of change in the duration of reproduction with increasing age to maturity may (i) remain unchanged, (ii) increase (adult life expectancy increasing more quickly, or decreasing more slowly with size than without the intervention), or (iii) reduce (increasing more slowly or decreasing more rapidly with size than without the intervention). In case (i), the proportionate change in fitness costs and benefits for worms maturing before or after  $\alpha^*$  will be

unchanged and the optimum age at maturity will be unaffected by the intervention. In case (ii), worms maturing after  $\alpha^*$  will enjoy a greater proportionate improvement in reproductive life than was the case with no intervention. Since the other components of fitness are unchanged, this means that increased fitness will now be achieved by worms maturing some time after  $\alpha^*$ , and such worms will be favoured by selection. In case (iii), the reverse occurs and selection will therefore favour earlier maturing worms.

As an example, consider parasites evolved to mature at the optimum age in hosts whose immune response increases in effectiveness with the size of adult worms. An intervention increasing adult mortality consistently for adult worms of all sizes would decrease the proportionate reduction in life expectancy for later maturing worms, whilst leaving unchanged the proportionate increase in fecundity, and reduction in chance of reaching maturity. This sort of intervention would favour worms with longer times to maturity.

The situation is further complicated because the direction of initial selection pressure as given by the sign of equation (5) need not indicate the overall direction of selection in cases where multiple local optima exist for the fitness function under an intervention,  $\omega_h(\alpha)$ . In such cases, one of which is illustrated in Fig. 3, the slope of  $\omega_h(\alpha)$  close to the original  $\alpha^*$  may not correspond to the change in  $\alpha$

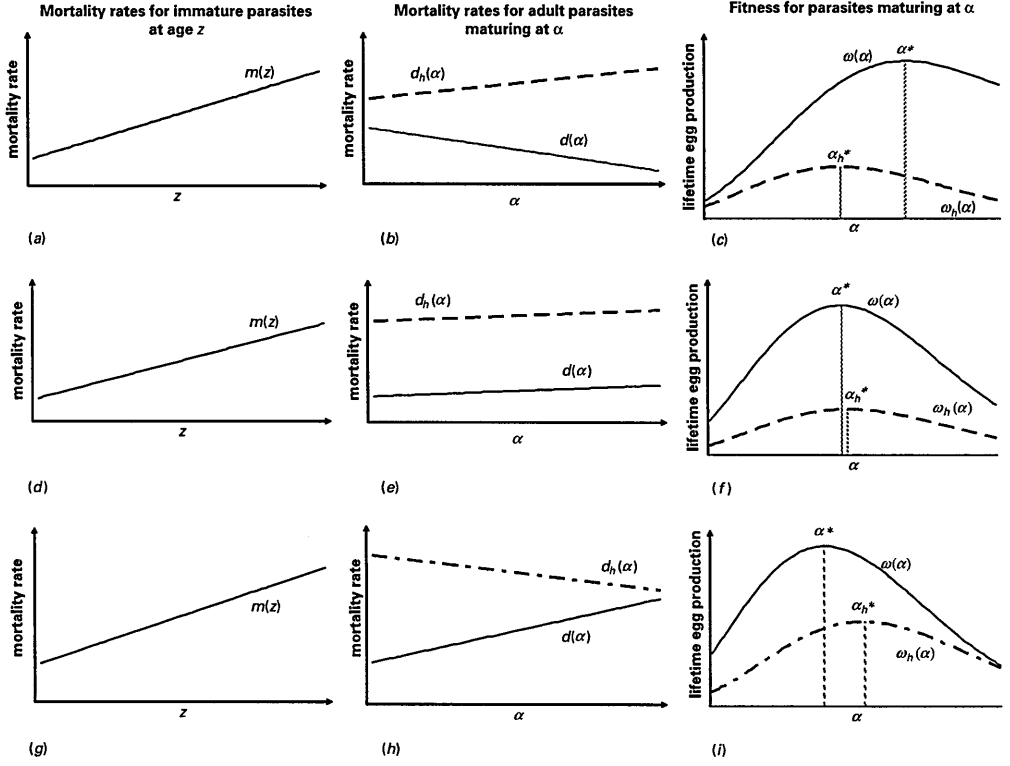


Fig. 2. Illustration of the effects of interventions increasing the adult mortality rate for parasites maturing at age  $\alpha$ . Panels (a) to (c) show an intervention which increases the proportionate rate at which adult mortality rate changes with age at maturity, resulting in a reduction in optimum time to maturity. Panels (d) to (f) show an intervention which keeps the same rate of increase in mortality rate, so that, with higher absolute mortality, there is a reduced proportionate rate of increase and hence an increased optimum time to maturity. Panels (g) to (i) show an intervention with reduced rate of increase in mortality rate, and also reduced proportionate rate of increase in mortality, as might result if an intervention more easily resisted by larger worms outweighed the effects of an immune response more easily evaded by smaller worms, giving an increased optimum time to maturity. Continuous lines show functions without the intervention, and dashed lines with the intervention.

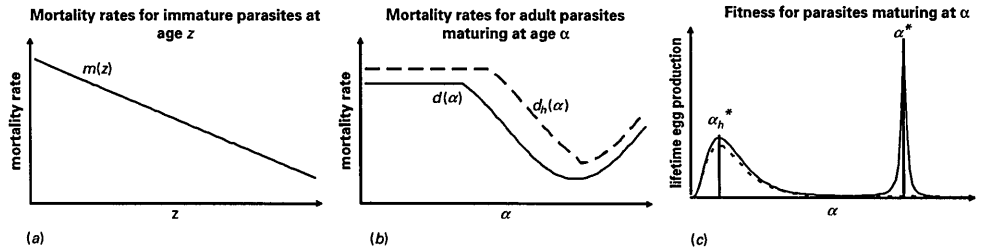


Fig. 3. Illustration of the effects of an intervention changing adult mortality in an example with multiple optima for the fitness function. Panel (a) shows the assumed pre-maturity mortality function, panel (b) shows the assumed post maturity mortality functions with and without intervention, and panel (c) shows the fitness functions with and without the intervention. The slope of the post maturity mortality function under the intervention is always less than or equal to that without the intervention, so initial selection pressure will favour increased time to maturity. However, the overall optimum now falls on a different peak of the fitness function and selection will in fact favour a lower value of  $\alpha$ . Continuous lines show functions without the intervention, and dashed lines with the intervention.

required to give the maximum achievable fitness. Outcomes in such cases will be unpredictable, depending upon specifics of starting conditions and the details of the functions involved.

#### Size-dependent mortality function with pulsed interventions

Drug treatments can arise as brief periodic events rather than on-going changes to mortality functions or fecundity parameters. Vaccine boosts (and some natural immunity processes) conceivably could do the same thing. The following assumptions and revised equations incorporate pulsed interventions, or interventions conferring transient changes in mortality, within the SDM model: (7) Dosing is periodic at a fixed interval,  $I$ . (8) Parasites are assumed to infect hosts randomly at a constant rate, and are thus equally likely to arrive at any time point during the interval between dosing events. (9) The proportion of parasites experiencing a second dose is assumed to be zero or very small for convenience of analysis. (Parameter values must be consistent with this assumption.). (10) The effect of the intervention on any given parasite is assumed to vary only according to whether the parasite is immature or adult, irrespective of size or age. (11) Between dosing events, mortality rates are in accordance with those given by  $m(z)$  and  $d(\alpha)$ .

Worms infecting a host during interval  $I$  can be divided into the following four groups. (A) Worms which die before the dosing event, without reaching maturity. These worms have zero fitness and thus do not contribute to the overall fitness function. (B) Worms which die before the dosing event, having reached maturity. These have fitness in accordance with the assumptions of the SDM model, but the post-maturity life expectancy must be the average for worms dying before  $I$ , not the overall post-maturity life expectancy. Fitness for worms in this category, arriving in the host at time  $t$ , is modelled by function  $f(t)$ . (C) Worms which survive until the dosing event, and are mature at the time of the dosing event. These worms will reproduce from maturity to age  $I - t$ , and then will either die in the dosing event, or will survive the dosing event and subsequently die according to the post-maturity mortality function. Fitness for worms in this category, arriving in the host at time  $t$ , is modelled by function  $g(t)$ . (D) Worms which survive until the dosing event and are immature at the time of the dosing event. These worms will either die in the dosing event before reproducing, or will survive to mature and reproduce in accordance with the SIM and SDM models. Fitness for worms in this category arriving in the host at time  $t$ , is modelled by function  $h(t)$ .

Using the symbols given in Table 1, the average fitness for worms in all categories arriving at

time  $t$  is given by

$$\begin{aligned}\omega_p(\alpha) &= \frac{\int_0^{I-\alpha} f(t)dt + \int_0^{I-\alpha} g(t)dt + \int_{I-\alpha}^I h(t)dt}{I} \\ &= \frac{c\alpha^\beta e^{-\mu(\alpha)}}{d(\alpha)} \left( 1 - \frac{H}{I} \left( D_m \frac{1 - e^{-d(\alpha)(I-\alpha)}}{d(\alpha)} + \alpha D_j \right) \right)\end{aligned}\quad (6)$$

The derivation of this expression is given in Appendix B.

In order to find the optimum value of  $\alpha$  under the pulsed intervention,  $\alpha_p^*$ , we require  $\omega_p'(\alpha_p^*) = 0$ , which, since  $\frac{c\alpha^\beta e^{-\mu(\alpha)}}{d(\alpha)}$  is non-zero, is equivalent to

$$\begin{aligned}0 &= \left( \frac{\beta}{\alpha_p^*} - \frac{d'(\alpha_p^*)}{d(\alpha_p^*)} - m(\alpha_p^*) \right) \\ &\quad \times \left( 1 + \frac{H}{I} \left( \frac{D_m(e^{-d(\alpha_p^*)(I-\alpha_p^*)} - 1)}{d(\alpha_p^*)} - \alpha_p^* D_j \right) \right) + \frac{H}{I} \\ &\quad \times \left( D_m \left( e^{-d(\alpha_p^*)(I-\alpha_p^*)} \left( 1 + \frac{d'(\alpha_p^*)}{d(\alpha_p^*)} \left( \alpha_p^* - I - \frac{1}{d(\alpha_p^*)} \right) \right) \right. \right. \\ &\quad \left. \left. + \frac{d'(\alpha_p^*)}{d(\alpha_p^*)^2} \right) - D_j \right)\end{aligned}\quad (7)$$

From this equation it is evident that, in addition to the detail of the underlying mortality functions  $m(z)$  and  $d(\alpha)$ , all the parameters associated with the pulsed intervention – the effectiveness of the treatment ( $D_m$ ,  $D_j$ ), the proportion of the host population treated ( $H$ ) and the interval between doses ( $I$ ) – have the potential to affect the evolution of time to maturity.

As for the SDM model, it is not possible to derive an explicit solution for  $\alpha_p^*$  for the SDMP model. However, again, the direction of the slope of the fitness function at  $\alpha^*$ , the optimum value of  $\alpha$  without the intervention, will give the direction of the initial selection pressure acting on time to maturity under the intervention. Since, from equation (4),  $\frac{\beta}{\alpha^*} - \frac{d'(\alpha^*)}{d(\alpha^*)} - m(\alpha^*) = 0$ , and since  $\frac{H}{I} \geq 0$ , the sign of the selection gradient at  $\alpha^*$  corresponds to the sign of  $s_p(\alpha^*)$ , where

$$\begin{aligned}s_p(\alpha^*) &= D_m \left( e^{-d(\alpha^*)(I-\alpha^*)} \left( 1 + \frac{d'(\alpha^*)}{d(\alpha^*)} \left( \alpha^* - I - \frac{1}{d(\alpha^*)} \right) \right) \right. \\ &\quad \left. + \frac{d'(\alpha^*)}{d(\alpha^*)^2} \right) - D_j\end{aligned}\quad (8)$$

It is clear that the sign of  $s_p(\alpha^*)$  will depend upon the detail of the mortality functions and the parameters of the pulsed intervention and hence that selection pressure may favour increased or decreased  $\alpha$  according to the specifics of  $m(z)$  and  $d(\alpha)$ , and the values for the intervention parameters,  $D_j$ ,  $D_m$

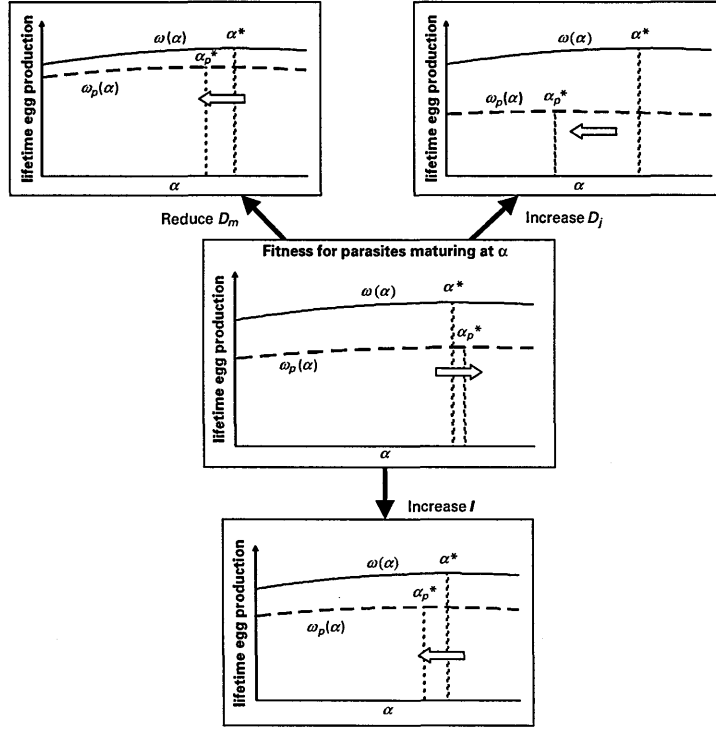


Fig. 4. Illustration of the effects of values for pulsed-dose model parameters on optimum time to maturity. From a given set of starting values, the direction of initial selection, for longer or shorter time to maturity can be changed by adjusting any of the three parameters, dosing interval,  $I$ , treatment mortality in immature parasites,  $D_j$ , and in mature parasites,  $D_m$ . Simple linear functions are assumed for  $m(z)$  and  $d(\alpha)$ , with negative slope for  $d(\alpha)$ . Continuous lines show the fitness function without intervention,  $w(\alpha)$ , dashed lines show the fitness function under pulsed intervention,  $w_p(\alpha)$ .

and  $I$ . Given this, it is also clear that increasing the pre-maturity mortality  $D_j$  will always act to reduce the strength of selection for increased time to maturity when  $s_p(\alpha^*) > 0$ , and to increase the strength of selection for reduced time to maturity when  $s_p(\alpha^*) < 0$ .

For example, Fig. 4 illustrates that the optimum age to maturity under a pulsed intervention may be either longer or shorter than that without intervention, depending upon the relative and absolute values of the parameters  $D_j$ ,  $D_m$  and  $I$ . Thus, within a given range of values for any two of these parameters, the direction of initial selection can be determined by the value of the third parameter. For instance, within a suitable range of values for  $I$  and  $D_m$ , changing the parameter  $D_j$  alone can change the direction of initial selection pressure. In each case, a limit may exist beyond which given values for one or more of these parameters fixes the direction of initial selection irrespective of the value of the others.

The proportion of hosts dosed,  $H$ , does not influence the direction of initial selection pressure. However, it does help to determine the size of the change from  $\alpha^*$  to  $\alpha_p^*$ , and can contribute to the

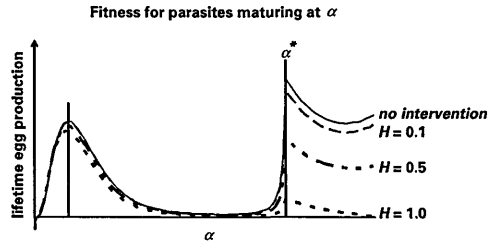


Fig. 5. Effect of  $H$ , proportion hosts dosed, on selection for time to maturity. In this example with multiple optima for the fitness function, although the selection gradient around  $\alpha^*$  is positive and initial selection favours increased time to maturity, increasing the value of  $H$  moves the global optimum to the earlier peak, giving overall selection in favour of a reduced time to maturity.

overall direction of selection pressure in cases with multiple solutions as illustrated in Fig. 5, where increasing  $H$  for a particular intervention produces very small changes in the values of  $\alpha$  at which the peaks of the fitness function fall, but ultimately causes the optimum value of  $\alpha$  to move from the

second to the first peak. In practice, the outcome of such a change would depend *inter alia* upon there being sufficient variation in  $\alpha$  within the parasite population to allow the transition between the two optima, given that most intervening values of  $\alpha$  would be selected against.

#### DISCUSSION

Nematode life history traits respond readily to selection (e.g. Paterson and Barber, 2007). Consequently, animal and human health programmes which alter nematode mortality schedules (almost always the aim of such programmes) can drive life-history evolution. For nematode age at maturity, a key life-history trait with important fitness consequences, we found that the resulting evolution could have variable outcomes. In some cases clinically beneficial evolution giving smaller, less fecund worms is likely. But in some cases, evolution prompted by animal and human health programmes could generate nematode life-histories which would be clinically detrimental: larger worms producing more eggs.

The simplest trade-off model of nematode age to maturity (Gemmill *et al.* 1999; Morand and Poulin, 2000), assumes size-independent mortality (SIM model above), and predicts that selection on age at maturity is primarily driven by juvenile mortality rates. Consequently, selection will always favour earlier maturity under interventions which increase mortality or reduce the fecundity gains associated with increased size. However, the models developed here show that when adult mortality rate changes with parasite size, then both adult and juvenile mortality rates influence the evolution of age at maturity. Critically, and unlike juvenile mortality, the effect of adult mortality on optimal age to maturity is not unidirectional. Analysis of equations (4), and (5) shows that enhanced adult mortality can select for earlier or later age to maturity. Thus it is possible for animal or public health interventions like immunisation programmes or widespread chemotherapy to promote either smaller less fecund worms or larger more fecund worms.

Which of these possible outcomes occurs will depend upon the biology of the parasite, the biology of the interactions between parasite and host immune system, and on the specifics of the health intervention applied. Predicting the outcome for any particular case requires knowledge of the pre- and post-maturity mortality functions, with and without the intervention. These are currently not known for any worm, and indeed they would be difficult to determine even where direct experimentation is possible. Furthermore, for pulsed interventions, the interval between doses, the proportion of hosts dosed, and juvenile and adult parasite mortality rates resulting from the treatment all also help to determine whether

selection will favour earlier or later maturing worms under the intervention. There are no simple generalities and indeed, given current levels of understanding, it is not even easy to speculate on which evolutionary outcomes are more likely.

Nonetheless, the complexity of this issue does not make it go away. Human interventions which change mortality schedules will exert selection pressure. In many cases, the resulting evolution in life-history traits will have little clinical significance, or will result in increased animal or public health. However, where, for example, the larval stage is much more pathogenic than the adult parasite, prolonging the time taken to reach adulthood may have undesirable clinical consequences. In such instances it would be important to take account of whether a given intervention strategy might be expected to select for a longer duration of larval stage, and plan accordingly.

In some instances, it may even be possible to avoid undesirable evolution. Often the selection pressures imposed by an intervention cannot be readily adjusted as, for example, with vaccine-induced immunity, although even here, the likely effects of stage or tissue-specific immunity could be investigated where there are several vaccine candidates being evaluated. For pulsed interventions, some elements, such as the time interval between doses, can readily be adjusted. Where such control is possible, rather than simply ameliorating selection for unwanted changes, it might be possible to specify an intervention to intentionally exert selection pressure in favour of a desirable change.

Detailed models developed to analyse specific cases could extend our models in a number of ways. For example, contrary to our assumption 11, worms which survive a dosing event may be damaged in some way and experience higher mortality rates, or have lower fecundity, than would otherwise be the case. This and other circumstances, such as seasonal life-cycles and dosing patterns might mean that worms are more likely to enter hosts early or late in the dosing cycle, contrary to our assumption 8. Certain combinations of dosing strategy and life-history may mean that a significant proportion of worms survive more than one dosing event, violating our assumption 9. Alternatively, density effects may mean that worms surviving a dosing event, or arriving in a host shortly after a dosing event, may experience lower mortality or higher fecundity than would otherwise be the case. We doubt that such complexities would alter our general conclusion that some interventions can select for clinically-detrimental worm evolution, but they might nonetheless be important considerations for evaluating the magnitudes of any such evolution in particular cases.

The relationship between mortality rate and age at maturity suggests that in an environment where

mortality rate showed variation, as would be expected within a normal host population, there would be benefits to the parasite in adjusting the age of maturity according to the mortality rate actually experienced or predicted in its individual host, provided the benefits of such flexibility outweighed the costs of achieving it. Such flexibility has been demonstrated experimentally for at least two nematode species (Guinnee *et al.* 2003). This may provide a means of testing our conclusions, by examining whether the changes flexibly adopted by worms under different mortality schedules, a system which should have evolved to maximise worm fitness, are consistent with the responses predicted by the models.

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Table 2. Equivalence of parameters used in the models discussed in Appendix A. A and M is from Anderson and May (1985), M and P, Morand and Poulin (2000) and SIM is the model of Gemmill *et al.* (1999) described in the current paper

A and M	Parameter Description	M and P	SIM
$k$	Parameter summarising aggregation of parasites within host population	not explicitly included	n/a
$s$	Proportion of females in parasite population	not explicitly included	n/a
$\Phi$	Mating function	not explicitly included	n/a
$\beta$	Transmission co-efficient between host and infective stages	$\beta$	n/a
$d_1$	Proportion of parasites entering host which survive to maturity	not explicitly included	n/a
$d_2$	Proportion of output transmission stages surviving to infective stage	assumed immediately infective	n/a
$N$	Host density	$H$	n/a
$\mu$	In-host parasite mortality rate arising from host death	$b$	part of $M_j$ and $M_a$
$\mu_1$	In-host parasite mortality rate arising from other causes	immature $\mu_L$ mature $\mu_p$	part of $M_j$ part of $M_a$
$\mu_a$	Free-living parasite mortality rate	$\mu_w$	n/a
$\lambda$	Fecundity/eggs per day	$\lambda = \alpha^a c$	$c\alpha^\beta$

#### APPENDIX A: MORAND AND POULIN MODEL

Morand and Poulin (2000) derived an alternative model for the relationship between parasite mortality rate and optimal time to maturity using  $R_0$ , the basic reproductive rate, based on explicit epidemiology, as follows;

$$R_0 = \frac{(\alpha^a)^c \beta H}{\alpha(\mu_w + \beta H) \left( \frac{1}{\alpha} + b + \mu_L \right) (b + \mu_p)} \quad (9)$$

giving

$$\alpha^* = \frac{-ca}{(ca-1)(\mu_L + b)} \quad (10)$$

with symbols as in Table 2. Equation (10) differs from equation (2). However, we show here that the two models give an equivalent solution for optimal age to maturity.

The derivation of equation (9) is based on a model by Anderson and May (1985),

$$R_0 = \frac{ks\Phi\beta d_1 d_2 N \lambda}{(\mu + \mu_1)(\mu_2 + \beta N)}$$

which separates the parasite mortality rate into two components, mortality of parasites within a living host, and parasite mortality through host death. The Anderson and May model also reflects a period of larval development outside the host prior to infectiousness, and a subsequent period of viability in the environment during which infective larvae may contact and infect hosts. Morand and Poulin (2000) ignored aggregation and implicitly assumed that all worms are hermaphrodite, so the parameters  $k$ ,  $s$ , and  $\Phi$  in the Anderson and May model can be ignored.

Morand and Poulin (2000) give the proportion of larvae infecting hosts which ultimately become adults within the host as  $\frac{1}{\alpha} \frac{1}{(\mu_L + b + \frac{1}{\alpha})}$ . This seems to be replicating the Anderson and May formula for the proportion of eggs produced which ultimately infect hosts, given by the probability of survival to infective stage  $\times$  life expectancy of infective larvae in the environment  $\times$  *per diem* transmission rate. However, this is not an appropriate representation of the process of in-host maturity where the transition from juvenile to adult occurs at age  $\alpha$  for all larvae surviving to age  $\alpha$ , not randomly at a given rate after age  $\alpha$  has been reached. In addition, the use of  $1/\alpha$  as the rate at which immature parasites become mature is inappropriate, since maturation does not happen randomly across all ages of immature parasites, but only to the proportion which have survived to age  $\alpha$ , and this would only be  $1/\alpha$  in the case where the in-host mortality rate among immature parasites was zero.

Using the parameters of the Morand and Poulin model, the amended formula for the proportion of immature parasites which survive a period of  $\alpha$  days from arrival in-host to reach maturity is  $e^{-(\mu_L + b)\alpha}$ .

Incorporating this means that equation (9) becomes

$$R_0 = \frac{\alpha^a c \beta H}{(\mu_w + \beta H)(b + \mu_p)} e^{-(b + \mu_L)\alpha} \quad (11)$$

giving

$$\alpha^* = \frac{-ca}{(\mu_L + b)} \quad (12)$$

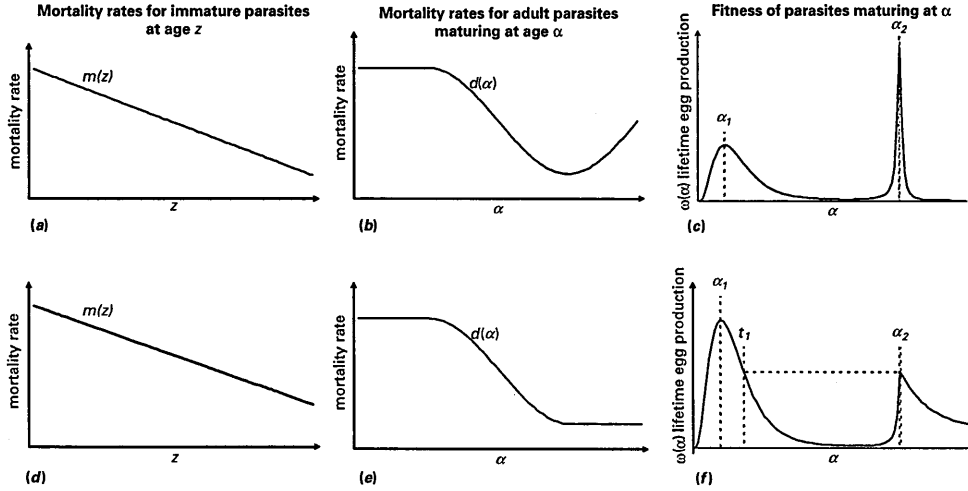


Fig. 6. Illustrations of multiple maxima for the fitness function (equation (3)). Mortality rates as a function of age for juveniles (left panels) and of age at maturity for adults (middle panels) generate the fitness functions shown in the right hand panels. The adult mortality function shown could arise if, for example, bigger worms are harder to kill and smaller worms are harder to detect. For (c), multiple local optima are found, with the global optimum falling on the later peak at  $\alpha_2$ . In (e), there are also multiple local optima, but the global optimum falls at  $\alpha_1$ , on the first peak. In this case, in the absence of lower limits on the time needed to physically achieve maturity, selection would favour maturity at  $\alpha_1$ . If minimum achievable time to maturity is between  $\alpha_1$  and  $t_1$ , selection will favour maturity at the minimum achievable age, and if the minimum achievable time to maturity is greater than  $t_1$ , then selection will favour maturity at  $\alpha_2$ .

Since  $(\mu_L + b)$  is the total mortality rate for immature parasites, equivalent to  $M_j$  in the SIM model, and  $ca$  is equivalent to  $\beta$  in the SIM model, equations (12) and (2) are equivalent.

#### APPENDIX B: ILLUSTRATION OF MULTIPLE MAXIMA FOR FITNESS FUNCTION

Fig. 6 gives examples of situations in which there can be more than one age to maturity associated with fitness maxima.

#### APPENDIX C: DERIVATION OF PULSED INTERVENTION MODEL

In this Appendix we derive expressions for the functions  $f(t)$ ,  $g(t)$  and  $h(t)$  introduced in section 3.2, and hence show that fitness is given by equation (6). For  $0 < t \leq (I - \alpha)$ , we have

$$f(t) = \text{probability of survival from } t \text{ to } t + \alpha \\ \times (1 - \text{probability of survival from } t + \alpha \text{ to } I) \\ \times \text{average life expectancy for worms dying between } t + \alpha \text{ and } I \\ \times \text{fecundity for worms maturing at age } \alpha$$

The average life expectancy post-maturity for worms born at time  $t$  which survive to time  $t + \alpha$

and die before time  $I$ , can be calculated from the definite integral on age  $q$ , measured from maturity, from 0 to  $(I - t - \alpha)$  of the proportion of such worms surviving to age  $q$  less the proportion which will survive to  $I$ .

Thus the average life expectancy post maturity, for worms born at time  $t$  which die between  $t + \alpha$  and  $I$  is

$$\frac{1}{1 - e^{-d(\alpha)(I - t - \alpha)}} \\ \times \left( \int_0^{I - t - \alpha} e^{-d(\alpha)q} dq - (I - t - \alpha)e^{-d(\alpha)(I - t - \alpha)} \right) \\ = \frac{1}{d(\alpha)} - \frac{(I - t - \alpha)e^{-d(\alpha)(I - t - \alpha)}}{1 - e^{-d(\alpha)(I - t - \alpha)}}$$

So

$$f(t) = c\alpha^\beta e^{-\mu(\alpha)} (1 - e^{-d(\alpha)(I - \alpha - t)}) \\ \times \left( \frac{1}{d(\alpha)} - \frac{(I - t - \alpha)e^{-d(\alpha)(I - t - \alpha)}}{1 - e^{-d(\alpha)(I - t - \alpha)}} \right) \\ = c\alpha^\beta e^{-\mu(\alpha)} \\ \times \left( \frac{1 - e^{-d(\alpha)(I - \alpha - t)}}{d(\alpha)} - (I - t - \alpha)e^{-d(\alpha)(I - \alpha - t)} \right)$$



For  $g(t)$ , we obtain, for  $0 < t \leq (I - \alpha)$

$g(t)$  = probability of survival from  $t$  to  $I$   
 $\times$  ((probability in undosed host + probability  
in dosed host but survives)  
 $\times$  (average life expectancy from  $I$ ) +  $I - \alpha - t$ )  
 $\times$  fecundity for worms maturing at age  $\alpha$   
giving

$$g(t) = c\alpha^\beta e^{-\mu(\alpha)} e^{-d(\alpha)(I-\alpha-t)} \\ \times \left( I - t - \alpha + \frac{1 - H + H(1 - D_m)}{d(\alpha)} \right)$$

For the function  $h(t)$  we find, with  $(I - \alpha) < t < I$

$h(t)$  = probability of survival from  $t$  to  $\alpha$   
 $\times$  (probability in undosed host + probability  
in dosed host but survives)  
 $\times$  average life expectancy from  $\alpha$   
 $\times$  fecundity for worms maturing at age  $\alpha$

which yields

$$h(t) = \frac{c\alpha^\beta e^{-\mu(\alpha)}}{d(\alpha)} \left( 1 - H + H(1 - D_j) \right)$$

The definite integrals of these functions over the relevant ranges for  $t$  give the following;

$$\int_0^{I-\alpha} f(t) dt = \frac{c\alpha^\beta e^{-\mu(\alpha)}}{d(\alpha)^2} \left( \left( d(\alpha)(I - \alpha) + 2 \right) e^{-d(\alpha)(I - \alpha)} \right. \\ \left. - 2 + d(\alpha)(I - \alpha) \right)$$

$$\int_0^{I-\alpha} g(t) dt = \frac{c\alpha^\beta e^{-\mu(\alpha)}}{d(\alpha)^2} \left( (2 - HD_m) \right. \\ \left. + \left( d(\alpha)(\alpha - I) + HD_m - 2 \right) e^{-d(\alpha)(I - \alpha)} \right)$$

$$\int_{I-\alpha}^I h(t) dt = \frac{c\alpha^{\beta+1} e^{-\mu(\alpha)} (1 - HD_j)}{d(\alpha)}$$

These functions are then combined to give the overall fitness function

$$\omega_p(\alpha) = \frac{1}{I} \left( \frac{c\alpha^\beta e^{-\mu(\alpha)}}{d(\alpha)^2} \left( \left( d(\alpha)(I - \alpha) + 2 \right) e^{-d(\alpha)(I - \alpha)} \right. \right. \\ \left. \left. - 2 + d(\alpha)(I - \alpha) \right) + \frac{c\alpha^\beta e^{-\mu(\alpha)}}{d(\alpha)^2} \left( (2 - HD_m) \right. \right. \\ \left. \left. + \left( d(\alpha)(\alpha - I) + HD_m - 2 \right) e^{-d(\alpha)(I - \alpha)} \right) \right. \\ \left. + \frac{c\alpha^{\beta+1} e^{-\mu(\alpha)} (1 - HD_j)}{d(\alpha)} \right) \quad (13)$$

This can be rearranged to give

$$\omega_p(\alpha) = \frac{c\alpha^\beta e^{-\mu(\alpha)}}{d(\alpha)} \\ \times \left( 1 - \frac{H}{I} \left( D_m \frac{1 - e^{-d(\alpha)(I - \alpha)}}{d(\alpha)} + \alpha D_j \right) \right)$$

which is equation (6).

## Essay

# How to Make Evolution-Proof Insecticides for Malaria Control

Andrew F. Read\*, Penelope A. Lynch, Matthew B. Thomas

## Summary

Insecticides are one of the cheapest, most effective, and best proven methods of controlling malaria, but mosquitoes can rapidly evolve resistance. Such evolution, first seen in the 1950s in areas of widespread DDT use, is a major challenge because attempts to comprehensively control and even eliminate malaria rely heavily on indoor house spraying and insecticide-treated bed nets. Current strategies for dealing with resistance evolution are expensive and open ended, and their sustainability has yet to be demonstrated. Here we show that if insecticides targeted old mosquitoes, and ideally old malaria-infected mosquitoes, they could provide effective malaria control while only weakly selecting for resistance. This alone would greatly enhance the useful life span of an insecticide. However, such weak selection for resistance can easily be overwhelmed if resistance is associated with fitness costs. In that case, late-life-acting insecticides would never be undermined by mosquito evolution. We discuss a number of practical ways to achieve this, including different use of existing chemical insecticides, biopesticides, and novel chemistry. Done right, a one-off investment in a single insecticide would solve the problem of mosquito resistance forever.

Indoor residual spraying (IRS) with insecticides continues to be a mainstay of malaria control, having been responsible for often spectacular reductions in disease incidence during the 20th century, including elimination of malaria from many countries [1–4]. More recently, insecticide-treated bed nets (ITNs) have become a leading tool for malaria control [4,5]. Major international efforts are currently underway to comprehensively control and even globally eradicate malaria, and these involve enormous up-

scaling of IRS and ITN use [6–10]. As in the last century, one of the major challenges to these new efforts is the evolution of insecticide resistance in *Anopheles* populations [1,2,11–18]. IRS spraying for malaria was responsible for resistance evolution in countries as diverse as Greece, Java, Haiti, and Sudan [17,19–21]. Insecticide-resistant mosquitoes were one of the main hurdles faced by the ultimately unsuccessful Global Malaria Eradication plan in the middle of last century [1,2,11,13,14,17,22]. Contemporary experience is that nothing has changed. For instance, a surge in malaria cases from 600/month to 2,000/month in KwaZulu-Natal, South Africa, at the end of last century was associated with pyrethroid-resistant *An. funestus* [23,24]. In a recent 24-village trial in Mexico, the frequency of pyrethroid-resistant *Anopheles* went from effectively zero to 20% after three years of IRS (Box 1) [25]. There are also serious concerns [16–18,26–31] and increasing evidence [32–34] that insecticides on bed nets will similarly drive resistance evolution.

Once a “resistance crisis” [26] occurs, where disease control fails because mosquito evolution has rendered an insecticide ineffective, options are few, not least because of the very limited insecticide arsenal available. Insecticides recommended for malaria control by the World Health Organization (WHO) represent just four classes of compound for IRS and just one class of compounds for ITNs [13,15]. Consequently, there is an increasing focus on resistance management strategies, whereby efforts are made to use existing insecticides in ways which can maximize the time period for which they provide useful disease control (what we hereafter refer to as the “useful lifespan” of a compound). Resistance management strategies

include the use of diverse insecticides in space and time (rotations and mosaics), insecticide mixtures, and restricting use to specific risk periods and locations [13,25,26,31,35–38]. Resistance management requires on-going surveillance [14,17] and a level of application management that is frequently problematic in regions where the malaria challenge is most severe. Moreover, techniques such as rotations and mixtures can be undermined by issues of cross resistance [13]. Indeed, given current restrictions on approved chemicals, there are virtually no options for resistance management for ITNs.

Consequently, there is now a concerted effort to identify new insecticidal compounds for use in malaria control [36,39]. On the face of it, this is desirable, but novel chemistry does not, in itself, provide a sustainable answer. All existing insecticides were “new” at some point, and there is

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**Abbreviations:** ADI age-dependent insecticide; DDT, dichloro-diphenyl-trichloroethane; FCM, feeding cycle model; ITN, insecticide treated nets; IRS, indoor residual spraying; IVCC, Innovative Vector Control Consortium; LLA, late-life-acting; PGM, population genetics model; TDI, time-delay insecticide; WHO, World Health Organization

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the very real danger that, as with the antimalarial drug treadmill [40], the search for products can become open ended as the efficacy of successful new compounds is, in turn, eroded by the evolution of resistance. Here we show that the natural history of the *Anopheles-Plasmodium* interaction makes possible an alternative strategy to deal with insecticide resistance: the development of insecticides with properties that retard and even entirely prevent the spread of resistance. An “evolution-proof” compound would provide sustainable control, render conventional resistance management strategies unnecessary, and completely avoid an insecticide treadmill.

### The Proposition

All current insecticides approved for ITNs or IRS kill extremely rapidly after contact, and some are also irritants that cause the mosquito to move away from the net or house and search for blood meals elsewhere. Where coverage is high (a requirement for effective control), insecticides greatly reduce malaria transmission, but their high lethality or interference with blood feeding also imposes intense selection for resistance. It is our contention that effective transmission reduction can be achieved while minimizing selection for resistance. To simplify the following discussion, we initially consider only the lethal effects of insecticides; we return to the irritant (excito-repellency) effects at the end.

Our argument derives from the following observations. First, female mosquitoes convert a blood meal into eggs and oviposit in appropriate water bodies before seeking the next blood meal. This gonotrophic cycle takes 2–4 d [41,42]. Females contact insecticides on bed nets during feeding attempts, or on house walls while resting immediately after the feed. Second, extrinsic mortality rates for the key vector species, even in the absence of any public health measures, are very high—on the order of 10% per day or 20–40% per gonotrophic cycle [41,42]. The consequence is that most females go through only a few gonotrophic cycles before they die. Third, after infecting mosquitoes, malaria parasites go through various developmental stages and very many replicative cycles before migrating to the salivary glands, from where they can be transmitted to

humans. The duration of this process (the sporogonic or extrinsic incubation period) depends on host, parasite, and environmental factors, but it is in the order of 10–14 d or 2–6 gonotrophic cycles in areas of high malaria transmission [41,42]. These facts together lead to one of the great ironies of malaria: most mosquitoes do not live long enough to transmit the disease.

These facts also mean that the majority of eggs a female will produce in her lifetime are laid in the window before malaria-infected mosquitoes can become dangerous to humans. Thus, in principle at least, public health advances can be achieved with minimal selection for resistance by an insecticide that kills after the majority of mosquito reproduction has occurred but before malaria parasites are infectious. Unlike in agriculture, the aim here is disease control, not necessarily insect control.

Below we consider how insecticides could be designed so as to kill only older mosquitoes, but we first compare the transmission control potential and the evolutionary properties of our proposed late-life-acting (LLA) insecticides with compounds like dichloro-diphenyl-trichloroethane (DDT), pyrethroids, and others currently in use (“conventional” insecticides). The first key question is whether LLA insecticides can offer significant reductions in malaria transmission.

### Control

To assess the malaria control potential of LLA insecticides, we followed others [42–44] in developing a simple feeding cycle model (FCM) that deterministically tracks discrete cohorts of mosquitoes through their gonotrophic cycles, where mosquitoes have fixed probabilities of becoming infected with malaria parasites and, in our case, exposed to insecticides. The background mosquito mortality rates and durations of sporogony used to parameterize the baseline model are the average of four *Plasmodium falciparum*-endemic sites, two in Nigeria, one in Tanzania, and one in Papua New Guinea [42]. These sites are intense foci of malaria transmission.

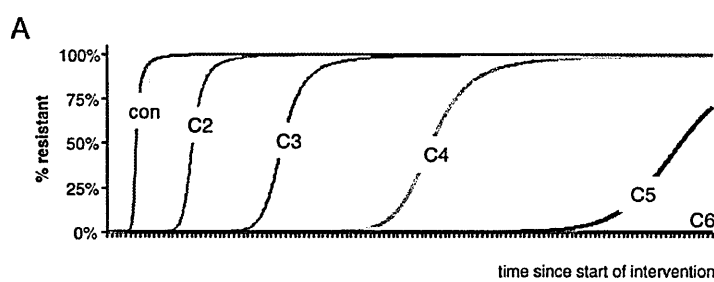
An LLA insecticide could disproportionately kill older mosquitoes in two ways. First, it might work some time after first exposure (a time-dependent killer), as might

be the case for an infectious agent. Second, the insecticide might be disproportionately effective against older mosquitoes, irrespective of time since contact (age-dependent killer), as might be the case if older insects are more physiologically vulnerable. In the following analysis, we consider this latter type of LLA insecticide, but our conclusions are unaltered in either case (Table S1).

The evolution of insecticide resistance is a practical problem only where insecticide coverage is high, which we take here to be 80%, a minimum target for coverage with IRS or ITNs [10]. For computational simplicity, we also assume that LLA insecticides have no impact on either total mosquito densities or the proportion of humans that are infectious. With these assumptions (and others, see Materials and Methods), we calculate that LLA insecticides killing mosquitoes that have reached 2 or more gonotrophic cycles will reduce the number of infectious bites by 99.2%. The corresponding figures for 3- and 4-cycle killers are 97.9% and 94.2%, respectively. These figures are highly encouraging, especially as they are minimum estimates: reductions in the number of infectious human cases following intervention will further reduce the number of infectious mosquitoes, as would higher or more-effective insecticide coverage and any effects on mosquito densities (more likely the earlier-acting the insecticide).

### Evolution

While fast-acting conventional insecticides can produce even more effective initial control (in our analysis, a 99.8% reduction in the number of infectious bites), they impose enormous selection for resistance by killing young female adults. The consequence is that spectacular initial mosquito control can last as little as a few years, thus providing very poor medium- to long-term disease control, as history has shown [22]. To analyze the evolutionary sustainability of LLA insecticides, we used fecundities calculated in our feeding cycle model as input into a discrete-time analog of standard population genetics models to track the spread of single-allele resistance through the population. Frequency of resistance in a population was calculated by assuming that



**B**

	Reduction in lifetime reproductive success of susceptible mosquitoes (%)	Relative fitness of resistant mosquitoes when insecticide present
conventional	84.6	6.49
2 cycle LLA	54.2	2.18
3 cycle LLA	34.5	1.53
4 cycle LLA	21.8	1.28
5 cycle LLA	13.4	1.15
6 cycle LLA	8.4	1.09

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**Figure 1.** Evolutionary Consequences of Insecticides That Are Highly Lethal Immediately after First Contact (Conventional Insecticides, like DDT and Pyrethroids, Con) and Hypothetical LLA Insecticides That Kill Mosquitoes from Their Second through Sixth Gonotrophic Cycles (C2–C6)

(A) Frequency of resistant mosquitoes through time. (B) Impact of insecticides on fitness of susceptible mosquitoes, and relative fitness of resistant mosquitoes in presence of insecticides, assuming no costs of resistance. LLA insecticides are a substantially less potent driver of the evolution of resistance than are conventional insecticides because of their substantially smaller impact on mosquito fitness. Note that when first deployed, four-cycle LLAs reduce the number of infectious bites by 94.2%. Two- and three-cycle killers remove more, but at cost of increased selection for resistance. We assume the control offered by five- and six-cycle killers, 76.6% and 57.1% of infectious bites removed, is too low to make them practicable (although absolute levels of control required will depend on local epidemiological context and the availability of other disease management tools). For model details and parameter values, see Materials and Methods.

resistance is dominant and ablates the mortality effects of the insecticide in question. We discuss the effect of relaxing the dominance assumption, and other assumptions, in Text S1.

With parameters as above, resistance spreads considerably more slowly for LLA insecticides than for conventional insecticides (Figure 1). This is because insecticides that kill on first contact will reduce mosquito lifetime reproductive success by about 85%. In contrast, insecticides that kill mosquitoes that have reached at least their fourth gonotrophic cycle eliminate just 22% of progeny (Figure 1). Thus, all else being equal, the fitness of a mutant resistant to conventional insecticides is 6.5 times

that of the susceptible wild type; the corresponding advantage for a four-cycle killer is just 1.28.

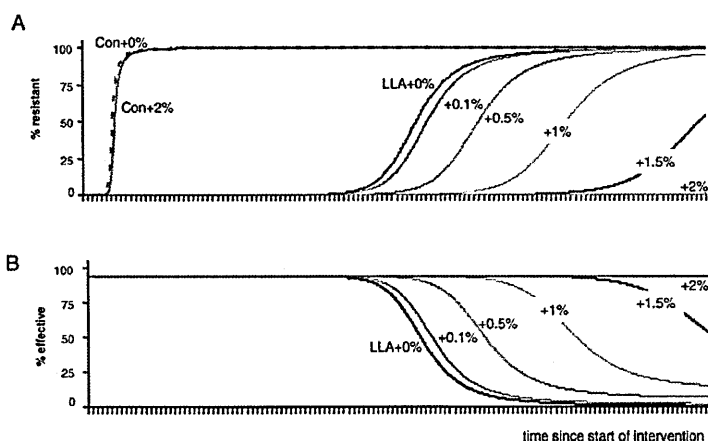
The evolution of resistance to LLA insecticides could be slowed even further if they were disproportionately effective against malaria-infected mosquitoes. This is because insecticides that are less likely to kill uninfected mosquitoes further relax selection for resistance without any loss of control. For instance, if we leave the probability that a four-cycle LLA insecticide would kill infectious mosquitoes unaltered but halve its likelihood of killing uninfected mosquitoes, the time taken for resistance to reach 50% frequency would increase by about half as much

again. A potentially useful side effect of disproportionate killing of malaria-infected mosquitoes would be to increase the selection pressure favoring malaria-resistant mosquitoes [45,46].

Importantly, resistance to LLA insecticides will not spread at all if there are nontrivial fitness costs to insecticide resistance. Reduced fitness of resistant insects in the absence of insecticides is frequently reported [47–49]. For *Anopheles*, costs of resistance have been seen in the laboratory [50,51] and, in the field, unexpectedly low frequencies of resistant homozygotes (e.g., [52]), and declines in resistance after withdrawal of causal insecticide (e.g., [18,25]) (see Box 1) point to substantial fitness costs. Costs of resistance have little impact on the evolution of resistance to conventional insecticides where the benefits of resistance are so high. The situation is, however, very different for LLA insecticides, where the fitness benefits of resistance (Figure 1) are very much lower. For LLA insecticides, resistance costs can outweigh resistance benefits, preventing resistance spreading at all, even when resistance alleles are present.

This argument follows from the evolutionary theory of aging [53–57]. The strength of selection declines with age. Beneficial genes that act late in life can fail to spread if they are associated with fitness costs earlier in life. This is because all individuals pay these costs, whereas only those few that survive to old age benefit. The theory of aging is well verified, not least in insects [58]. Senescence does occur in mosquito populations, and in *Anopheles* is detectable around the age at which mosquitoes can first become infectious to humans [59–62]. Thus, natural selection has not been strong enough to favor beneficial alleles that would act around the same time as would a putative resistance allele against a late-life insecticide.

The inclusion of even modest costs of resistance substantially slows the rate at which resistance to LLA insecticides spreads in a population, thus considerably prolonging the effectiveness of malaria control (Figure 2). Importantly, it is also possible to maintain the initial levels of control forever. For the particular parameter values used here, costs of resistance, which accrue as an additional daily mortality rate of 3.4%, would



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**Figure 2.** Evolutionary Consequences and Control Effectiveness of Insecticide Where There Are No Costs of Resistance (+0%) or Where the Costs of Resistance Accrue as Additional Daily Mortality Rates as Shown

(A) Frequency of resistant mosquitoes through time. (B) Control effectiveness through time, where 0% effectiveness is the situation prior to the insecticide application, and 100% is complete absence of infectious bites. Plotted values are for a conventional insecticide (Con) or an LLA insecticide that kills mosquitoes on contact during or after their fourth gonotrophic cycle (a 4C LLA in Figure 1). Beyond the duration of our simulations, the LLA insecticide eventually fails even for a 2% cost of resistance (green line); for the parameter values used here, complete evolution-proofing occurs at 3.4%. For model details and parameter values, see Materials and Methods.

render a four-cycle LLA insecticide completely evolution proof: this is the point at which the fitness gains of resistance, which benefit only a few, are outweighed by the fitness costs of resistance, which are paid by all. Thus, in principle at least, it is possible to create an insecticide that would provide effective malaria control yet never be undermined by the evolution of resistant mosquitoes.

The cost of resistance required to get evolution proofing is lowered for LLA insecticides which are disproportionately effective against malaria-infected mosquitoes (Figure 3). For instance, a four-cycle LLA insecticide, which is half as likely to kill uninfected mosquitoes, requires a cost of resistance of just 2.3% to be completely evolution proof. Strikingly, if its effectiveness against uninfected mosquitoes was just 10% of what it was against infected mosquitoes, complete evolution proofing would occur at a resistance cost of just 0.9%, a cost which would be barely measurable. An LLA insecticide that kills only malaria-infected mosquitoes is completely evolution proof for vanishingly small costs of resistance (0.43%).

We are aware of only one quantitative estimate of the relative fitness of

resistant mosquitoes in the field. This comes from the non-malarial vector *Culex pipiens*, following 40 years of organophosphorous (OP) insecticide spraying in Southern France [48,63]. There, the fitness of individuals homozygous for a resistance mutation relative to sensitive homozygotes is 0.63–0.72 (discussed further in Text S1). Using our model to calculate lifetime fecundity of mosquitoes experiencing various mortality costs of resistance in the absence of treatment, we find that the relative fitness associated with the highest cost of resistance required to get complete evolution proofing, 3.4% additional mortality, is 0.78; the corresponding figures for the 2.3% and 0.9% additional mortality described above are 0.84 and 0.93, respectively. Similar figures are obtained if we assume the costs of resistance accrue as reduced fecundity rather than reduced adult survival (unpublished data). Thus, the costs of resistance required to achieve complete evolution proofing are not out of line with those seen in nature.

### Product Options

The foregoing analysis argues that new insecticides for malaria control should minimize impact

on mosquito lifetime reproductive output while also minimizing the number of infectious mosquitoes. The achievement of this goal ideally requires insecticides that kill late in life, that are disproportionately effective against malaria-infected mosquitoes, and for which resistance carries fitness costs. This approach, which will retard the spread of resistance alleles (possibly forever) even when they are already present in a population, should complement or even replace strategies aimed solely at delaying the initial origin of resistance, since these latter strategies often have no effect when resistance eventually becomes established in a population.

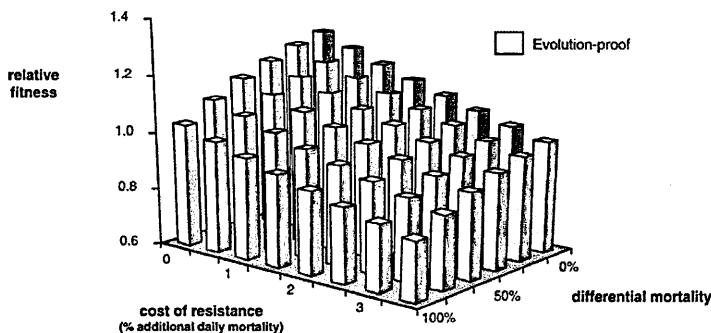
We are unaware of any attempts to evaluate potential insecticides for these properties, but it is possible to imagine a range of approaches or modes of action that would achieve late-life killing. For example, cumulative exposure to ordinarily sublethal doses of an insecticide over multiple feeding cycles could result in the death of older mosquitoes. Alternatively, formulation techniques such as microencapsulation could provide a means for slow release of an insecticide over time. Similarly, age-dependent mortality could be achieved by exploiting the fact that in *Anopheles*, metabolic detoxification activity declines with age [29,64]. This decline may be a natural consequence of senescence and explain why *Anopheles* become more susceptible to DDT, malathion, and pyrethroids with increasing age [64–68]. It is also easy to imagine compounds that would act disproportionately on mosquitoes with advanced malaria infections. Malaria parasites impose large metabolic costs on mosquitoes [69–73], either directly via competition for resources, or indirectly by prompting costly immune responses. These costs are likely to increase as the malaria infection progresses, both as a consequence of the increasing parasite burdens as replication proceeds, and as blood and other meals become progressively less successful as the mouthparts become blocked with sporozoites [74]. Metabolically stressed insects should be more vulnerable to normally sublethal doses or compounds.

An even more radical possibility is that there may be formulations or deployment strategies that would convert existing insecticides into

evolution-proof LLA insecticides. As noted above, DDT, pyrethroids, and malathion are disproportionately efficacious against mosquitoes that are old enough to transmit malaria [64–68]. Doses lower than those currently recommended may therefore be insufficient to kill younger mosquitoes but fatal to older, near-infectious mosquitoes. If so, existing insecticides could be evolution-proofed by changing concentrations delivered in the field, even where resistance is currently spreading in a population.

The evolutionary benefits of an LLA insecticide apply irrespective of the resistance mechanism involved, but the greatest benefits accrue for compounds against which resistance is the most costly. Resistance to conventional insecticides involves target site alterations, metabolic detoxification, and behavioral avoidance [2,12,13]. It seems highly likely that the fitness costs of resistance will depend on the mechanisms involved. In other insects, there is evidence that fitness costs depend on the insecticide, and for some but importantly not all, the costs can clearly be negligible or degrade through time as modifiers spread [63,75]. Explicit deployment of compounds against which resistance is costly would be a novel approach and would also assist traditional resistance management strategies.

There may also be ways of achieving evolution-proof insecticides by means other than chemicals. For example, fungal biopesticides are already known to generate the required phenotypes. These insecticides are based on oil-formulated spores of entomopathogenic fungi applied to surfaces on which adult mosquitoes will rest after blood feeding [46,76,77]. Although still at a research stage, they have proven to be very effective malaria transmission blockers in the laboratory [76] and can be delivered in African houses [77]. Fungal biopesticides work as time-dependent late-life insecticides, killing the insect 7–14 d post-contact [46,76–79]. They are also disproportionately effective against malaria-infected mosquitoes [76]. Other biocontrol agents such as *Wolbachia* [80] and densoviruses [81] have a similar potential to disproportionately target older mosquitoes [82], and hence are potentially immune to the evolution of host resistance.



doi:10.1371/journal.pbio.1000058.g003

**Figure 3. Fitness of Resistant Mosquitoes Relative to Susceptibles for an LLA Insecticide for Various Costs of Resistance and Differential Efficacy against Malaria-Infected Mosquitoes**

When relative fitness is greater than 1, resistance spreads, when relative fitness is less than 1, resistance can never spread, even when present in a population (complete evolution-proofing). Plotted values are for an LLA insecticide which kills mosquitoes on contact during or after their fourth gonotrophic cycle; these remove 94.2% of infectious mosquitoes when first deployed. Differential mortality is the proportionate reduction in mortality for uninfected mosquitoes compared to malaria-infected mosquitoes. Costs of resistance accrue as additional daily mortality rates. Relative fitness for conventional insecticides is 6.5 (Figure 1), which is little affected by costs resistance (see text). For model details and parameter values, see Materials and Methods.

Moreover, nothing in our arguments actually requires compounds that kill mosquitoes. Critical is that older, infectious mosquitoes be prevented from biting humans. Killing them is one way of doing this, but analogous arguments would apply to products which, late in life, have other transmission-blocking effects, such as interference with host-seeking behavior, flight, or blood feeding propensity. Sublethal effects like these must have pronounced fitness consequences for mosquitoes but, as with lethality, these need not result in strong selection for resistance so long as they impact in later life. Fungal biopesticides reduce feeding propensity as infection progresses [76,83]. Irritancy is an important feature of the protection offered by some existing chemical insecticides like pyrethroids, because it drives mosquitoes out of houses and in search of other hosts [33]. For highly anthrophilic species, like *An. gambiae*, evolution-proofing an irritant would require that it be selectively excito-repellent to older mosquitoes. For vector species that are not particularly anthrophilic, an insecticide that achieved irritancy without lethality would impose negligible selection for resistance at any age if the fecundity and survival of mosquitoes feeding on nonhuman hosts was no lower.

## Complications and Possible Downsides

Exploiting the ideas advocated above requires that criteria used to evaluate insecticides for malaria control be broadened beyond those currently now in use. Current minimum target product profiles required by the WHO Pesticide Evaluation Scheme for Phase 1 (laboratory) testing of insecticides for ITN and IRS use are 80% mortality up to 24 h post-exposure in young (2–5 d post-emergence) adult female *Anopheles* [84,85]. These thresholds, little changed since the 1960s [86], are used by the WHO to determine which insecticides to recommend to national authorities, and consequently by others to determine candidate compounds for inclusion in product development portfolios (for example, the Innovative Vector Control Consortium; [http://www.ivcc.com/workwithus/application\\_process/irs.htm](http://www.ivcc.com/workwithus/application_process/irs.htm); accessed 4 March 2009). However, these “young-kill” criteria will result in the use of insecticides that impose near maximal selection for resistance. Minimizing that selection while still providing malaria control requires the use of insecticides and application protocols that impose marked reductions in transmission potential while simultaneously minimizing reductions in mosquito fitness. Assessing that requires exposing



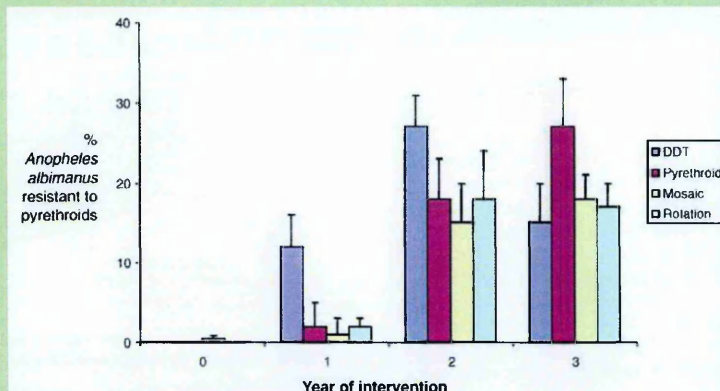
### Box 1. A Contemporary Example of the Selection of Insecticide Resistance by Indoor Residual Spraying

Some of the best data on the impact of malaria control insecticides on resistance in *Anopheles* come from the Pacific Coast of Chiapas, Mexico [25,92,93]. In this region, agricultural use of insecticides around mosquito breeding sites together with indoor residual spraying of DDT for malaria control resulted in high levels of resistance to organochlorines, organophosphates, carbamates, and pyrethroids by the end of the 1970s. In the 1980s and '90s, DDT continued to be used for malaria control, and DDT resistance remained at high levels. However, the agricultural use of insecticides declined markedly, so that by the mid-1990s, resistance to all other classes of insecticides had regressed to the point where it was barely detectable in standard WHO bioassays [93]. Genetic and biochemical analyses confirmed that, nonetheless, several known resistance alleles remained in these populations.

In the latter half of the 1990s, a 24-village IRS trial was conducted, aimed at evaluating the effect of contrasting resistance management strategies on the evolution of resistance [25,92,93]. This trial was prompted by rising concerns that the practice of using insecticides until resistance became a limiting factor was rapidly eroding the number of insecticides available for malaria control. Villages were assigned to one of four treatments of repeated cycles of house-spraying: (i) two spray applications per year of DDT, or three applications per year of (ii) a pyrethroid, (iii) a spatial mosaic of an organophosphate and a pyrethroid, or (iv) an annual rotation of an organophosphate, a pyrethroid, and a carbamate.

Over the three years of the trial, pyrethroid resistance increased markedly in the mosquito populations in all villages, irrespective of insecticide treatment (Figure 4). Thus, spray campaigns targeting mosquitoes in an age-independent manner can very rapidly drive resistance evolution when relevant alleles are present in a population. Presumably, the majority of mosquitoes in all villages would have been resistant had the trial continued a few more years. This trial was well resourced and monitored, so that the insecticide coverage achieved was likely to be as high as is practically possible, and thus representative of an IRS campaign conferring maximal possible malaria control.

Resistance measures based on forcefully exposing mosquitoes to insecticide, such as the WHO bioassays used to generate the data in the figure, likely under estimate epidemiologically relevant resistance because they can not assay important forms of resistance such as behavioral avoidance. Moreover, even resistance to direct exposure can be due to many different mechanisms and there can be many genetic variants in any one biochemical pathway. Thus, the contribution of any particular allele to overall resistance varies substantially. In this trial, levels of cytochrome P<sup>450</sup>, a major determinant of resistance to pyrethroids, were maintained at high levels only in villages sprayed solely with pyrethroids. In villages sprayed with DDT or subject to the rotation scheme, cytochrome P<sup>450</sup> levels declined below detectability [25]. This suggests that cytochrome P<sup>450</sup>-mediated resistance can be managed by switching to a different insecticide class, but also that such switches need not limit resistance at the whole-insect level (Figure 4). It is our contention that evolution-proofing is possible for all resistance mechanisms, even where they already exist in a population, by targeting older mosquitoes.



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**Figure 4.** Evolution of Insecticide Resistance over Three Years of Indoor Residual Spraying in Coastal Southern Mexico.

Pyrethroid resistance was assessed using WHO susceptibility bioassays before intervention (year 0) and over three years of different insecticide spraying regimes. Redrawn from [25].

cohorts of young and old mosquitoes to insecticides, and analyzing life-long life tables, propensity to blood feed and, critically, fecundity, all ideally done with malaria-infected mosquitoes going through regular gonotrophic cycles.

Such experiments are not technically demanding, but they are logistically challenging, so that it would be impractical to do such tests for thousands of candidate compounds. However, for a limited number of

promising candidates, such tests are feasible [46,76–79]. Candidates could be chosen in a number of ways. First, highly lethal compounds already at an advanced stage of development (or even registered) could be tested at lower concentrations for LLA properties. Second, known compounds, possibly rejected in previous screens because of slow speed of kill, could be revisited. Third, other product evaluation criteria such as likely cost,

environmental safety, and potential for cross resistance could be used to preselect candidates for LLA testing from among the thousands of compounds currently tested in standard protocols. With lower lethality as a requirement, many more compounds might become feasible public health tools. We note that when it costs >US\$175 million to bring a new compound into use [10], even substantially higher initial development

costs for one LLA product look good against the costs of having to develop a second and third conventional insecticide (potentially ad infinitum if malaria can not be eradicated or controlled some other way). They also look good against the indefinite implementation costs and logistic constraints of resistance management strategies such as rotations or mosaics, which are currently being investigated as a means to prolong the life of existing, fast-acting insecticides once resistance is present (Box 1).

One side effect of the highly lethal insecticides currently in use for malaria control is that they also kill nontargets, such as nuisance mosquitoes and bedbugs. This side-effect is believed to help with household compliance and uptake [3,37,87], at least before the nontargets also evolve resistance. LLA insecticides would not have these immediately beneficial side effects (although a product with differential impact against primary targets and secondary targets is a possibility). As such, LLA insecticides would essentially be community-level interventions, like transmission-blocking vaccines, with the associated issues of user take-up. Accordingly, it may be that LLA insecticides will require delivery mechanisms that provide some degree of personal protection against nuisance insects, like bed nets, or imaginative, culturally-sensitive delivery systems and education programs that facilitate adoption irrespective of immediate personal relief from biting insects.

The late-life killing insecticides we are proposing here work because of the time *Plasmodium* takes to develop in mosquitoes. Could these insecticides select more rapidly developing parasites [82,88]? They might, but the short lives of mosquitoes must already be imposing intense natural selection for shorter extrinsic incubation periods, a selection pressure that must be further exacerbated by conventional insecticides. The apparent lack of response to this selection implies that significant fitness gains result from prolonged development [46,89], gains which presumably accrue through increased infectiousness [74]. It might be that LLA insecticides would add sufficient additional selection to offset these, but if it did, the resulting evolution would presumably generate substantially less-fit malaria parasites.

Further investigation of this possibility is certainly warranted; in the meantime we note that the hypothetical evolution of significantly less-infectious parasites must be of less public health significance than the observed failure of existing insecticides in the face of resistance evolution.

For equivalent levels of coverage (at least lower than 100%), conventional insecticides will always give better control initially, before any resistance evolution. This disparity widens as coverage drops (unpublished data). Indeed, if only poor coverage can be achieved, the control benefits of LLA insecticides may be negligible. However, in that case, the need for them is also negligible, because resistance evolution is much less of a problem at low coverage, where insecticides of any type will impose weaker selection for resistance. LLA insecticides come into their own when coverage is high, an explicit aim of ITN and IRS programs, particularly in intense transmission areas. At high coverages, sustained reductions in transmission of ~95% by an LLA insecticide will quickly outweigh the even higher reductions that are initially possible with conventional insecticides once resistance against the latter inevitably spreads. Even LLA insecticides which fall short of being completely evolution-proof will minimize the evolutionary pressures that otherwise rapidly erode the efficacy of conventional insecticides. Very much slower rates of increase of resistance give more time for surveillance to detect resistance problems (or less frequent surveillance to provide the same warning), and more time to react. Lower selection pressures can also translate into many decades of additional effective control, which from a practical control perspective may be essentially infinite.

### Concluding Remarks

Somewhat ironically, given that all the insecticides currently in use in the public health sector derive from products developed for the agricultural sector, the long-term sustainability of LLA insecticides could be further enhanced precisely because they are likely to have little utility in agriculture. The linkage between public health and agricultural use of insecticides plagues public health use of insecticides like DDT and pyrethroids, where agricultural

applications are one of the major drivers of resistance in vector populations [13,17,90]. This linkage could be broken by choosing LLA insecticides which could not be profitably reformulated for agricultural use, and for which there is no cross-resistance with existing agricultural pesticides. Moreover, restricted to the much smaller public health arena, any environmental impact of LLA insecticides would also be substantially reduced. However, an insecticide exclusive to public health would be unable to exploit the financial drivers promoting investment in agricultural insecticides, and so would need an artificially constructed market of the sort necessary to encourage the pharmaceutical industry to invest in malaria vaccines.

Our argument that public health insecticides can be evolution-proofed will not generalize to all vector-borne diseases, but it may be applicable to others with extrinsic incubation periods that approach the life spans of their vectors. Such diseases may include dengue, filariasis, West Nile virus, Japanese encephalitis, onchocerciasis, and Chagas disease. Novel technologies directed against a variety of disease vectors, such as those exploiting genetic modification of mosquitoes and selfish genetic elements, could also be immune to the evolution of host resistance if they are late-life acting.

The Global Malaria Action Plan (GMAP) [10] has laudable ambitions of spraying 172 million houses annually, and distributing 730 million insecticide-impregnated bed nets by the year 2010. If implemented with existing insecticides, this program will impose unprecedented selection for resistance. The historical record [22], and theory (e.g., Figure 1) shows that the medium-term prognosis for the insecticides currently in use is inescapably poor. Transitioning to an LLA insecticide strategy could see the benefits of the massive GMAP effort sustained, and could maintain for the long term the contribution of several key vector control tools to the goal of eradication. Failure to address evolution now runs the risk of replaying history [22]: operational disaster and a derailing of the whole malaria control agenda.

### Materials and Methods

The aim is to compare the relative effects of various hypothetical



insecticides on (i) malaria transmission and (ii) evolution of resistance. Age-structured models of vector-borne diseases are notoriously difficult to parameterize, but because our aim is comparison of insecticides (our aim is theoretical proof-of-principle), and not absolute rates or amounts, considerable simplification is possible.

Our analysis consists of two parts: a static deterministic feeding cycle model (FCM) similar to those used by others [42–44], and a population genetics model (PGM). The FCM tracks, for each gonotrophic cycle over the lifetime of a mosquito (up to a maximum of ten cycles), probabilities of survival, contact with insecticides, frequency and ages of malaria infections, and the number of eggs laid. Incorporation of relevant mortality assumptions allows the FCM to assess the impact of a particular insecticide on the average lifetime number of infectious bites per mosquito and the average fecundity per mosquito. The PGM then uses the survival, infectious bite, and fecundity figures from the FCM for each class of mosquito to calculate, for the population as a whole, the relative frequency of resistant mosquitoes (our measure of resistance evolution) and the average number of infectious bites per mosquito (our measure of control), over a series of time periods (each equivalent to the length of one gonotrophic cycle), using standard population genetics approaches.

The FCM makes the following assumptions.

1. Mosquitoes bite humans randomly and uniformly.
2. Malaria-infected mosquitoes never become uninfected.
3. The proportion of humans who are infectious is constant.
4. A variety of parameters do not change over successive gonotrophic cycles: (i) the background mosquito mortality rate (what Smith and McKenzie [44] call “force of mortality”), which is considered as a constant per-capita daily death rate (i.e. there is no senescence), (ii) the probability of taking a blood meal and (iii) the probability of feeding on a human.
5. Conventional insecticides are instant kill.

LLA insecticides are envisaged to kill in either of two ways: (i) when they contact a mosquito after she has been through a fixed number of gonotrophic cycles, e.g., a four-cycle age-dependent insecticide (ADI) kills mosquitoes that have been through four or more cycles; or (ii) a fixed number of cycles after first contact, as might be the case for an infectious agent, e.g., a four-cycle time-delay insecticide (TDI) kills mosquitoes four cycles after initial contact. We have modeled both; the values we report are for ADIs. In Table S1, we show that ADIs and TDIs have equivalent effects. [Note that a mode of action for an LLA insecticide could also be via bioaccumulation, where lethal concentrations of an insecticide are finally achieved after repeated contacts over course of a mosquito’s life. We have not explicitly modeled that mode of action].

The non-mathematical description of the model, considering ADIs only, is as follows. Female mosquitoes are followed from successful emergence through ten gonotrophic cycles. In each cycle, the probabilities of survival are tracked through the processes of host seeking, feeding, resting, finding an oviposition site, and laying. For each cycle, the proportion of mosquitoes that acquire a malaria infection, bite whilst infectious for malaria, and successfully lay eggs is also recorded. The mosquito may die whilst searching for a host, with a probability arising from the time spent searching and the background mortality rate. If she survives searching, she then attempts to feed on a human with a given probability, and on a nonhuman with one minus that probability. She may die whilst attacking the host immediately before or immediately after feeding, with probabilities calculated from the underlying risk of death when attacking a host, and the probability of encountering an insecticide (conventional or ADI) that kills on contact. Of those that successfully feed on a human host, females carrying a mature malaria infection give an infectious bite, whilst those so far uninfected may become infected, with a fixed probability. Those that survive feeding may then die during resting with a probability calculated from the time spent resting, and the background mortality rate. Those surviving resting may die whilst searching for a resting

site, again depending on time and relevant mortality rates, and survivors may then die whilst attempting to lay, either before or after laying, with fixed probabilities. The tracked values give the proportion of mosquitoes surviving, biting, and laying in each cycle.

The variables and parameters used in the FCM to generate the figures reported in the paper are given in Table S2 with equations in Protocol S1. Differential mortality of malaria-infected and uninfected mosquitoes was calculated by applying only a proportion of the mortality associated with a given treatment to individuals not infected with malaria. The full mortality is applied to malaria-infected individuals. The model was implemented in Microsoft Excel [91].

The PGM makes the following assumptions:

1. Adult mosquito population size is constant.
2. Mosquitoes do not complete more than ten gonotrophic cycles.
3. The genetic make-up of mating males in any cycle is the same as that calculated for newly hatched mosquitoes in that cycle.
4. Males of all resistant/susceptibility genotypes are equally likely to mate successfully.
5. Females mate once only, in their first cycle, as is the norm [45].
6. Number of eggs produced per laying female is unaffected by egg paternal genotype.
7. Genotypes of emerging adults joining the population are in the same proportions as the genotypes of the generation of eggs from which they hatch.
8. Resistance is dominant, as can be the case [52].
9. Costs of resistance are dominant.
10. The proportion of infectious humans is constant.

Variables and parameter values for the PGM are given in Table S3 and associated equations are given in Protocol S2. The model uses survival probabilities from the FCM to calculate the initial age structure within the susceptible phenotypes in the population. The resistant allele is assumed initially to be present in heterozygotes, forming a very small proportion of the population, as detailed in Table S3. Subsequent

spread of the allele reflects the age-linked survival probabilities for susceptible mosquitoes in the presence of the treatment and for resistant individuals, as well as the age-linked fecundity of each, all calculated in the FCM. The model, implemented in Microsoft Excel [91], analyses the changing status of the population for 1,290 sequential discrete time periods, each equivalent to the length of one feeding cycle.

Further discussion of model assumptions and sensitivity analyses are given in Text S1, together with additional analysis of the merits of the approach.

## Supporting Information

### Protocol S1. Mathematical Details of the Feeding Cycle Model

Found at doi:10.1371/journal.pbio.1000058.s001 (19 KB PDF).

### Protocol S2. Mathematical Details of the Population Genetics Model

Found at doi:10.1371/journal.pbio.1000058.s002 (19 KB PDF).

### Table S1. The Equivalence of Time-Dependent and Age-Dependent Insecticides

Found at doi:10.1371/journal.pbio.1000058.s001 (10 KB PDF).

### Table S2. Variables and Parameters of the Feeding Cycle Model

Found at doi:10.1371/journal.pbio.1000058.s002 (24 KB PDF).

### Table S3. Variables and Parameters of the Population Genetics Model

Found at doi:10.1371/journal.pbio.1000058.s003 (19 KB PDF).

### Text S1. Additional Discussion of Assumptions

Found at doi:10.1371/journal.pbio.1000058.s003 (24 KB PDF).

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# Prospective malaria control using entomopathogenic fungi: comparative evaluation of impact on transmission and selection for resistance

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## Abstract

**Background:** Chemical insecticides against adult mosquitoes are a key element in most malaria management programmes, but their efficacy is threatened by the evolution of insecticide-resistant mosquitoes. By killing only older mosquitoes, entomopathogenic fungi can in principle significantly impact parasite transmission while imposing much less selection for resistance. Here an assessment is made as to which of the wide range of possible virulence characteristics for fungal biopesticides best realise this potential.

**Methods:** With mathematical models that capture relevant timings and survival probabilities within successive feeding cycles, transmission and resistance-management metrics are used to compare susceptible and resistant mosquitoes exposed to no intervention, to conventional instant-kill interventions, and to delayed-action biopesticides with a wide range of virulence characteristics.

**Results:** Fungal biopesticides that generate high rates of mortality at around the time mosquitoes first become able to transmit the malaria parasite offer potential for large reductions in transmission while imposing low fitness costs. The best combinations of control and resistance management are generally accessed at high levels of coverage. Strains which have high virulence in malaria-infected mosquitoes but lower virulence in malaria-free mosquitoes offer the ultimate benefit in terms of minimizing selection pressure whilst maximizing impact on transmission. Exploiting this phenotype should be a target for product development. For indoor residual spray programmes, biopesticides may offer substantial advantages over the widely used pyrethroid-based insecticides. Not only do fungal biopesticides provide substantial resistance management gains in the long term, they may also provide greater reductions in transmission before resistance has evolved. This is because fungal spores do not have contact irritancy, reducing the chances that a blood-fed mosquito can survive an encounter and thus live long enough to transmit malaria.

**Conclusions:** Delayed-action products, such as fungal biopesticides, have the potential to achieve reductions in transmission comparable with those achieved with existing instant-kill insecticides, and to sustain this control for substantially longer once resistant alleles arise. Given the current insecticide resistance crisis, efforts should continue to fully explore the operational feasibility of this alternative approach.

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## Background

The impressive reductions in global malaria burden achieved this century by chemical insecticides against adult mosquitoes could be eroded by insecticide-resistant mosquitoes [1-6], just as they were last century [7]. In principle, the evolution of insecticide resistance could be considerably slowed and perhaps prevented altogether by vector control aimed at killing only older mosquitoes, so-called late-life action (LLA) [8]. Malaria parasites in a mosquito host take at least nine days to develop to a stage which can be transmitted to a human via an infectious bite [9]. Since mortality in wild mosquito populations is high, the majority of eggs are produced by young mosquitoes. Thus, a vector-control treatment which kills only older mosquitoes could remove infected mosquitoes before they can transmit malaria whilst only impacting the reproductive success of only the relatively few mosquitoes that survive to old age. This would dramatically reduce transmission while exerting only weak selection for resistance.

One option for an LLA vector-control measure is entomopathogenic fungi [10]. Naturally occurring strains of two fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, are already in commercial use for agricultural applications and have been shown to infect and kill mosquitoes in laboratory and field settings. Fungal spores can be picked up by mosquitoes following contact with treated surfaces, and so could be used against mosquitoes in indoor residual spray (IRS) programmes, or delivered via traps, curtains or netting [11-16].

A wide variety of mortality schedules can be induced in *Anopheles* by entomopathogenic fungi [17]. In some cases, all mosquitoes can be killed within a few days; in others, background mortality rates can be barely altered. This virulence variation depends on isolate [11], dose [18] and malaria-infection status [15,18], see also [19]. Lethality can also be increased by genetically modifying fungal isolates [20-22].

If fungal entomopathogens are to realize the potential of the LLA approach to sustainable malaria control, candidate biopesticides need to be chosen which balance reductions in parasite transmission (maximized by high fungal virulence) with resistance management (maximized by low fungal virulence). Here a mathematical model is used to ask which virulence phenotypes best achieve this balance. The intention is to guide the development of target product profiles. The possible efficacy of fungal biopesticides in IRS campaigns is compared with that of pyrethroid-based insecticides now in widespread use. Pyrethroids are highly lethal if contacted by a mosquito, but they also have a strong excito-repellency effect, which can drive away mosquitoes before they receive a lethal dose [23-25]. There is evidence that fungal spores do not repel mosquitoes [26], raising the prospect

that, for IRS, fungal biopesticides might more effectively reduce transmission than pyrethroid-based technologies currently in use.

## Methods

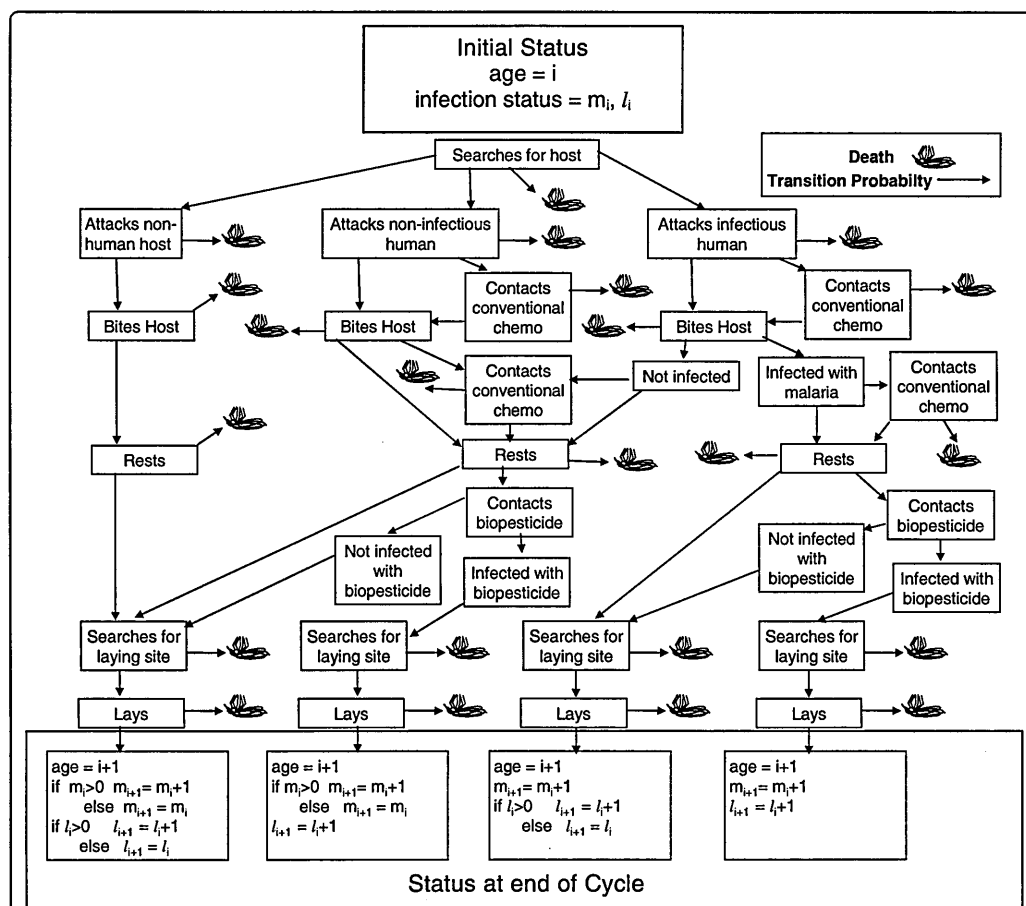
### The model

Many malaria transmission models already exist [27], but most do not capture the detailed timings and probabilities of infection, infectiousness, reproduction and mortality over the mosquito lifespan which are key to assessing whether LLAs can provide a useful balance of transmission control and low selection for resistance. In order to encompass these elements, a model has been developed with two separate components, a markovian, deterministic, feeding cycle model (FCM) which calculates survival, egg-laying and infectious bite values during the lifetime of an adult mosquito, and a population model (PM) which tracks the population-level spread of resistance alleles and corresponding loss of transmission control. The model is a development of a simpler version previously used to evaluate putative chemical LLAs [8]. Other modelling frameworks used to assess the LLA approach are heuristically useful but lack sufficient detail to define target virulence schedules [28-30].

### The feeding cycle model

The FCM calculates survival, egg-laying and infectious bite values across a series of discrete adult age classes for a specified type of mosquito (e.g., susceptible) subjected to a given intervention (e.g., a particular fungal biopesticide at a particular coverage). Each sequential age class is defined as lasting for the average length of one gonotrophic cycle. Use of the mosquito feeding cycle as the basis for age-structured analyses of mosquito populations is well established [31-34].

The FCM tracks possible states and transitions through each age class ( $i$ ), applying survival, exposure and infection probabilities (Figure 1). Infection status for a biopesticide ( $l$ ) or malaria ( $m$ ), is zero for no infection, otherwise equal to the age of the infection. State changes depend on the preceding state, the passage of time, mortality rates and the probabilities of certain events, such as contacting a biopesticide when resting after a human blood meal. For example, for a case analysing the effects of a fungal biopesticide, a mosquito commencing its fourth cycle with an infectious, three-cycle-old malaria infection, and no fungal infection, will spend a defined period of time searching for a host, with an associated probability of dying from background mortality while it does so. It will then attack a host, with a given probability that the selected host will be a non-infectious human, a malaria-infectious human, or non-human, and a given probability of being killed whilst attacking the host before biting. If it survives to bite, and if the host is



**Figure 1 FCM model structure for one feeding cycle.** Calculation of possible outcomes for feeding cycle  $i+1$  of the model, assuming status when commencing cycle  $i+1$  is age =  $i$ , malaria status =  $m_i$ , fungal infection status =  $l_i$  with  $l_0=0$  and  $m_0=0$ . Each arrow represents a probability calculated by the model. If the malaria infection status (number of cycles since infection) of a mosquito when biting is greater than the development time of the malaria parasite in the mosquito, then 'bites host' after attacking a human host is recorded as an infectious bite.

human, this is recorded as an infectious bite. There is then a given probability that it is killed by the host after biting. If it is not killed, it begins a period of resting, during which, if the chosen host was human, it has a fixed probability of encountering and being infected by the fungus, as well as a given probability of dying from background mortality before leaving to search for an egg-laying site. During the search for an egg-laying site there is a given probability that the mosquito may die from background mortality, or from the effects of its newly acquired fungal infection. If it survives searching for an egg-laying site it may die before or after laying with given probabilities. If still alive at the end of the cycle, it

begins its fifth cycle with an infectious, four-cycle-old malaria infection, and a one-cycle-old fungal infection. For a case analysing the effects of a conventional instant-kill chemical pesticide, the analysis would include a probability of contacting the pesticide after biting the host, and a probability of death, assumed to be instant, resulting from that contact, with zero probability of contacting a biopesticide.

Both conventional instant-kill and delayed-action biopesticides offer public health benefits by reducing the numbers of mosquitoes that survive to give infectious bites in a treated population. Clearly the extent to which a reduction in infectious bites maps to reduced transmission

and reduced numbers or severity of malaria cases in a human host population involves many complex, context-specific factors. For comparative purposes, however, it is assumed that in a given context, a given reduction in infectious bites will generate the same reduction in malaria transmission and malaria morbidity and mortality irrespective of the type of intervention from which it results. For generality, therefore, the comparative public health benefits of the insecticides considered in this analysis are all evaluated based on the reduction in infectious bites which they provide. This is quantified in the FCM for a given phenotype by calculating RAIB, the proportionate reduction in the average number of infectious bites per mosquito per lifetime (AIB), defined as

$$\text{RAIB} = 1 - \frac{\text{AIB with treatment}}{\text{AIB without treatment}}$$

Assuming that the rate at which newly maturing adults join a population is constant through time, and that the size of the human host population is unaffected by the intervention being assessed, RAIB is equal to the proportionate reduction in the entomological inoculation rate (EIR), the number of infectious bites experienced per person per unit of time.

To evaluate mosquito fitness, the average number of eggs produced per mosquito per lifetime is used as a proxy for lifetime reproductive success (LRS). The selection coefficient, the proportionate fitness benefit of resistance to a given intervention, is calculated as Selection Coefficient =  $1 - \frac{\text{LRS for specified mosquito type with intervention}}{\text{LRS for susceptible mosquitoes without intervention}}$ . A selection coefficient of zero means no selection pressure in favour of resistance, with higher selection coefficients indicating increasingly strong selection for resistance.

Formulating these key variables in relative terms minimizes the sensitivity of the conclusions to parameter values that are independent of the vector-control treatment or mosquito phenotype being evaluated.

The primary definitions of the FCM are given in Table 1, and its main features are detailed below. A detailed derivation of the FCM is given in Additional file 1: Appendix A. Baseline parameter values used in the analysis are summarized in Table 2.

The probability that a mosquito contacts and is affected (killed or infected) by a conventional or biological insecticide after biting a human host is input as a single 'coverage' value, incorporating the probabilities of being in a treated property, of contacting the pesticide, and of being affected by the pesticide during contact. It is assumed that physical constraints on the proportion of surfaces and internal areas treated will apply equally to conventional and fungal insecticides, and that for mosquitoes contacting treated surfaces, biopesticides

can potentially offer rates of infection equivalent to the rates of mortality generated by conventional insecticides. The latter assumption is supported by field trials showing >86% infection of mosquitoes entering outdoor bait boxes [36], 76% infection in experimental huts with fungus-impregnated eave curtains [13], and laboratory trials showing >95% infection from treated clay pots [14] or exposure to treated clay tiles [11].

The average number of eggs laid in a given cycle, by mosquitoes surviving to the start of that cycle,  $F_i$ , is calculated as

$$F_i = \frac{\left( \sum_{m=0}^{i-1} \sum_{l=0}^{i-1} f_{i,m,l} V_{i,m,l} \right)}{V_i}$$

This provides the basis for the evaluation of relative fitness using a comparison of values for  $\phi$ , lifetime egg

production, representing LRS,  $\phi = \sum_{i=1}^{\lambda} F_i V_i$ .

Comparative levels of transmission control are assessed using  $u$ , the average number of infectious bites

per mosquito lifetime,  $u = \sum_{i=1}^{\lambda} I_i V_i$ .

The average number of infectious bites during cycle  $i$  per mosquito surviving to the beginning of cycle  $i$ ,  $I_i$ , is calculated as

$$I_i = \frac{\sum_{m=D}^{i-1} \sum_{l=0}^{i-1} q_{i,m,l,2} V_{i,m,l} + q_{i,m,l,3} V_{i,m,l}}{V_i} \quad i > D$$

The average probability of survival from start of cycle  $i$  to start of cycle  $(i + 1)$  is  $S_i$ , with

$$S_i = \frac{\left( \sum_{m=0}^{i-1} \sum_{l=0}^{i-1} s_{i,m,l} V_{i,m,l} \right)}{V_i}$$

## The population model

The PM tracks susceptible and resistant phenotypes over a sequence of time periods for a population subject to a given vector-control treatment. The key outputs, calculated for each time period, are the proportion of the population with resistant and susceptible phenotypes and the overall reduction in infectious bites across the population compared to a susceptible population with no vector-control treatment.

The variables and parameters for the PM are described in Table 3, and baseline values used in the analysis are summarized in Table 4.

A detailed derivation of the model is given in Additional file 2: Appendix B. In brief, the PM works in discrete time periods, each equivalent to the length of one gonotrophic cycle, with recruitment of newly emerged adult mosquitoes

**Table 1 Variables and parameters for the feeding cycle model**

Variable or Parameter	Symbol	Comments and Constraints
Time, measured in whole units equal to average length of sporogonic cycle, from infection of mosquito by malaria to cycle from which mosquito gives infectious bites	$D$	input $0 < D$
Number of age classes included in analysis	$\lambda$	
Cycle number (identifies specific cycle in the $\lambda$ cycles over which probabilities are tracked in the FCM)	$i$	$0 \leq i \leq \lambda$
Malaria status, the number of whole or partial cycles since infection with malaria	$m$	$0 \leq m \leq \lambda$ , $m = 0$ means not infected
Biopesticide infection status, the number of whole or partial cycles since infection with biopesticide	$l$	$0 \leq l \leq \lambda$ , $l = 0$ means not infected
Average number of eggs laid in cycle $i$ by mosquitoes surviving to the start of cycle $i$	$F_i$	
Average lifetime number of eggs laid per mosquito	$\phi$	
Average number of eggs laid in cycle $i$ , by mosquitoes starting cycle $i$ with malaria status $m$ and biopesticide status $l$	$f_{lm,i}$	$m < i < l$
Average probability of survival from start of cycle $i$ to start of cycle $i+1$	$S_i$	
Average probability that a mosquito starting cycle $i$ with malaria status $m$ and biopesticide status $l$ , will survive to start of cycle $i+1$	$s_{lm,i}$	$m < i < l$
Average probability of a mosquito being alive at start of cycle $i$	$V_i$	
Average probability of a mosquito being alive, with malaria status $m$ and biopesticide status $l$ , at start of period $i$	$v_{lm,i}$	$m < i < l$
Probability that a mosquito alive at start of cycle $i$ with malaria status $m$ and biopesticide status $l$ , survives and bites host type $h$ in cycle $i$	$q_{lm,ih}$	$m < i < l$
Type of host attacked	$h$	$h=1$ , non-human $h=2$ , non-infectious human $h=3$ , infectious human
Average number of infectious bites in cycle $i$ per mosquito alive at the start of cycle $i$	$I_i$	
Average lifetime number of infectious bites per mosquito	$u$	

treated as occurring at the start of each time period. For each sequential time period, the proportion of the population comprised by each genotype in each age class is calculated, reflecting the genotypes of new adult recruits and the survival of adults in each age class from the preceding period. This is then used to calculate the proportion of the total population in time period  $n$  with homozygous recessive ( $G_{3,n}$ ) and heterozygous ( $G_{2,n}$ ) genotypes, from which  $R_n$  is calculated, the proportion of the population with a resistant phenotype in period  $n$ , with  $R_n = G_{3,n} + G_{2,n}d$ . Dominance is actioned by the value of  $d$ , which is 0 when resistance is assumed recessive, and 1 when it is assumed to be dominant.

Results from the FCM are used by the PM to calculate the average number of infectious bites per mosquito in the population during each time period. From this  $Q_n$ , the number of infectious bites given by the population as a whole relative to those given by an untreated population, can be calculated for each time period as  $Q_n = \frac{M_n W_n I}{q}$ .

**Assumptions**

The model does not attempt to capture the effects of mutational processes or stochastic demographic effects on the origin and initial spread of very low numbers of

resistance alleles, and so it is assumed that resistant phenotypes are already established at a low frequency in the population at the start of the analysis. Resistance involves a single gene and a simple dominant/recessive process. Moreover, it is assumed that the size and age structure of the population at the start of the PM analysis is that achieved after sustained use in a susceptible population of the insecticide being evaluated, that there is no immigration or emigration, and the proportion of each genotype in the new adults joining the population matches that in the eggs from which they originate. Density dependence is assumed to occur at the mosquito larval stage and the number of adult mosquitoes recruited to the population per unit of time remains constant.

All model parameters are age-independent, apart from background mortality rates and the action of age-linked pesticides, with incremental mortality from fungal biopesticide infection varying according to the number of days since infection. Conventional insecticides affecting a susceptible individual are assumed to be instantly fatal. Mosquitoes choose human hosts at random, and the model does not capture feedback between numbers of infectious bites and the proportion of human hosts with infectious malaria. Malaria-infected mosquitoes never become uninfected. All feeding cycles are of equal duration and mosquitoes bite once in each cycle. All eggs



**Table 2 Values used in FCM for this analysis**

Variable or Parameter	Symbol	Value	units
Background instantaneous mortality rate for mosquito age <i>i</i>	$r_{B,i}$	11.75% <sup>1</sup>	per day
Length of gonotrophic cycle	<i>w</i>	2.85 <sup>1</sup>	days
Time spent host searching and feeding during a cycle	<i>b</i>	1.26 <sup>5</sup>	days
Time spent finding oviposition site and laying during a cycle	$\phi$	1.26 <sup>5</sup>	days
Length of resting period (days)	$\eta$	0.32 <sup>5</sup>	days
Proportion human population infectious for malaria <sup>4</sup>	<i>p</i>	4.28% <sup>1</sup>	
Probability attacks non-human host	<i>H</i>	0.17 <sup>1</sup>	
Probability killed when attacking host before biting	<i>a</i> <sub>1</sub>	.05 <sup>6</sup>	
Probability killed when attacking host after biting (excluding mortality from insecticide treatments)	<i>a</i> <sub>2</sub>	.05 <sup>6</sup>	
Probability becomes infected with malaria when biting infectious human host <sup>4</sup>	<i>M</i>	1.00	
Number of eggs laid per successfully laying mosquito per cycle	<i>L</i>	100 <sup>2</sup>	eggs
Time, measured in whole units equal to length of gonotrophic cycle, from infection of mosquito to cycle from which mosquito gives infectious bites	<i>D</i>	3 <sup>3</sup> Based on 10.78 <sup>1</sup> days	cycles
Baseline probability that mosquito contacts and is killed by conventional instant-kill chemical insecticide (CC) whilst resting after biting human host	<i>k</i>	0 for cases not assessing use of CC 0.8 for cases assessing use of CC	
Baseline probability that mosquito contacts and is affected by delayed action pesticide whilst resting after biting human host	<i>X</i>	0 for cases not assessing use of delayed action pesticide 0.8 for cases assessing use of delayed action pesticide	
Number of age classes included in analysis	$\lambda$	10	cycles

1.Averages taken from four geographic locations [31]. Results using individual geographic data sets are expected to give qualitatively equivalent results. Limited sensitivity analysis was consistent with this assumption, so use of the average figures was considered adequate for the present analysis.  
2.Since we are only interested in comparative values, the absolute value for the number of eggs per lay is immaterial, 100 has been used as a convenient normalised value.  
3.The number of cycles assumed for sporogonic development is calculated from the average number of days for sporogonic development and the average number of days per gonotrophic cycle, rounded down to give a whole number of cycles. This is a conservative assumption with respect to the amount of EIR reduction calculated for given fungal virulence parameters.  
4.The data set used provides a total probability of acquiring a malaria infection when biting a human host. This has been used as the value for parameter *p*, with *M*=1.00, to give the appropriate combined probability, *Mp*.  
5. Assumes c.11.1% of every cycle is spent resting (8 hours in a 72 hour cycle), with the rest of the gonotrophic cycle divided equally between laying and feeding.  
6. Estimated 10% mortality per feeding attempt [35], divided equally between pre- and post-bite.

laid are of equal quality and viability. The analysis assumes that malaria infection produces no effects on behaviour, background mortality or fecundity in infected mosquitoes, and fungus-infected mosquitoes that survive and lay eggs are assumed to lay as many eggs at each laying event as uninfected individuals.

Mosquitoes are assumed to contact the chemical or biopesticide when resting after biting a human host, reflecting an application method essentially consistent with IRS. Avoidance behaviour such as outdoor feeding and outdoor resting is not reflected in the coverage values for susceptible mosquitoes since it comprises a method of resistance.

**Analysis**

A number of fungal strains have now been tested in laboratory mosquito populations, and a wide range of mortality characteristics have been observed around the basic pattern of initial fungal growth and development followed by an increase in observable mosquito

mortality [11,15,16,18,37]. This suggests that most virulence profiles are potentially available, and in a search for generalizable results this analysis therefore uses highly simplified virulence mortality characteristics, defined by two parameters, ‘initiation day’, the time from infection to the onset of fungus-induced mortality, and the daily mortality rate from that point (Figure 2).

Fungal biopesticides can also impact mosquito feeding propensity and flight capacity in the days before mosquito death [11]. A mosquito which no longer attempts to feed or to lay eggs is effectively dead from the perspectives of fitness and disease transmission. For the purpose of the model therefore, ‘mortality’ encompasses cessation of feeding and reproduction, as well as actual death.

**Results**

**Coverage and virulence**

The proportionate reduction in EIR generated by use of a biopesticide is affected by fungal virulence and coverage

Table 3 Variables and parameters for the population model

Variable or Parameter	Symbol	Comments & Constraints
Period number (periods over which the population is tracked)*	$n$	$0 < n$
Dominance of resistance allele	$d$	dominant $d = 1$ recessive $d = 0$
Genotype (normal allele $s$ , resistant allele $r$ )	$g$	$(s,s) \ g = 1$ $(s,r) \ g = 2$ $(r,r) \ g = 3$
Proportion of total population having genotype $g$ at start of period $n$	$G_{g,n}$	
Proportion of the population resistant at start of period $n$	$R_n$	
Average number of infectious bites per mosquito in population in period $n$	$M_n$	
Size of initial population (susceptibles in the presence of treatment) as proportion of base population (susceptibles without treatment)	$J$	value from FCM
Population size in period $n$ as proportion of initial population size	$W_n$	
Average infectious bites during one time period from an untreated population	$q$	value from FCM
Number of infectious bites from treated population during time period $n$ , expressed as a % of the number of infectious bites during one time period from a susceptible population without treatment,	$Q_n$	Chosen measure of control
Number of periods between egg-laying and adult emergence	$\Phi$	Input

\*As for the FCM, the duration of one gonotrophic cycle is used as a unit of time. For convenience we use 'cycles' to refer to mosquito age and 'periods' to refer to the sequential time periods for which values are calculated in the PM.

(Figure 3). For a given level of coverage, similar levels of EIR reduction are achieved by various combinations of the two parameters used to summarize virulence (initiation day and mortality rate (Figure 2)). Unsurprisingly, the longer a fungus takes to initiate mortality, the greater the subsequent mortality rate has to be to maintain a given level of reduction in EIR. There are limits to the EIR reductions that can be achieved at low virulence and/or low coverage.

For equivalent reductions in EIR, selection for resistance is best minimized by high coverage with late initiation day, high mortality rate biopesticides. For example, the lowest selection coefficient associated with a 90% RAIB at 80% coverage is 21%, with day 9 initiation and a 91% mortality rate. At 50% coverage the lowest selection coefficient available in combination with 90% RAIB is 40%.

Table 4 Values used in the population model for this analysis

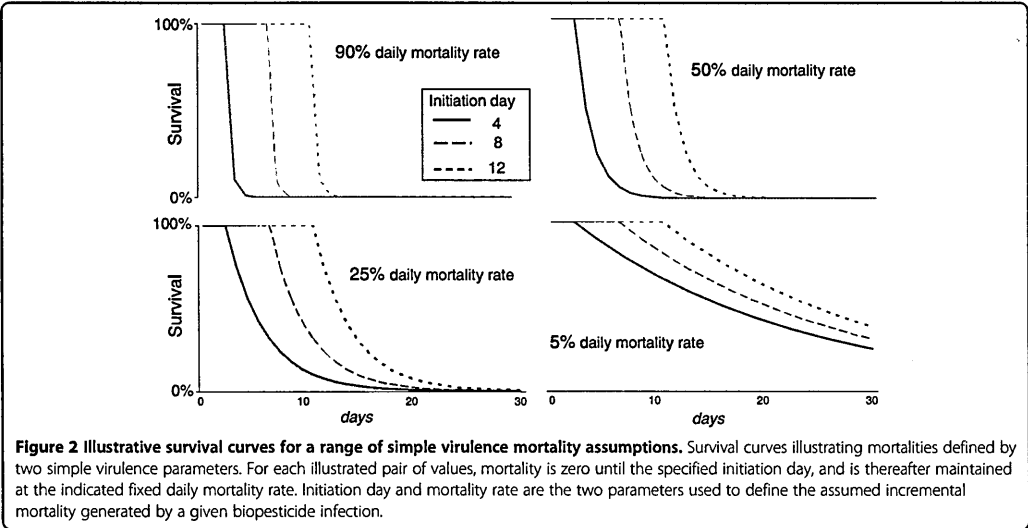
Variable or Parameter	Symbol	Value
Proportion of total population having genotype $g$ at start of period 1	$G_{g,1}$	$G_{1,1} = 1 - G_{2,1}$ $G_{2,1} = 10^{-9}$ $G_{3,1} = 0$
Dominance of resistance allele (0=recessive, 1=dominant)	$d$	$d = 1$
Number of periods between egg-laying and adult emergence	$\Phi$	3
Fitness factor for males with genotype $g$	$f_g$	$f_1=f_2=f_3=1.00$

All other input values use results calculated by the FCM.

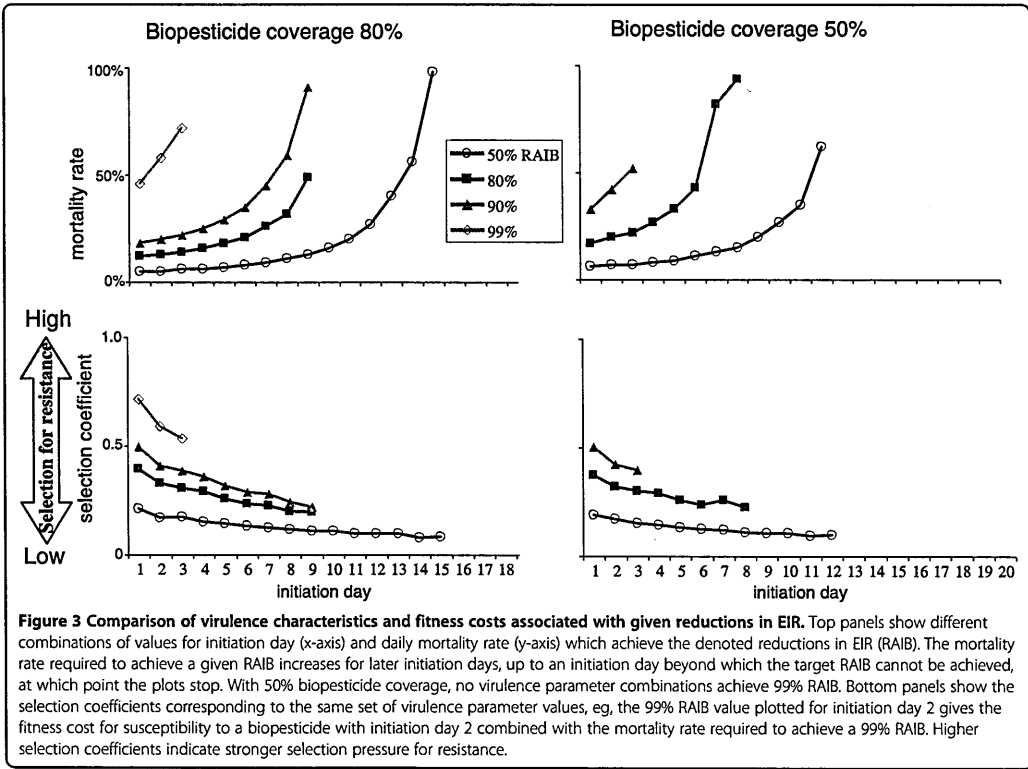
The temporal dynamics of EIR reduction and resistance evolution are shown in Figure 4. Predictably, more virulent biopesticides give better population-level reductions in EIR to begin with, but they then drive the evolution of resistance more rapidly. The speed of resistance evolution is more sensitive to the timing of mortality onset than to the incremental mortality rate.

The evolutionary dynamics and resulting pattern of control failure differ markedly for different insecticides even when they give identical reductions in EIR in the pre-evolutionary phase (Figure 5). Conventional instant-kill chemical insecticide (with coverage adjusted to achieve the same initial control) fails first. The longest time to product failure is offered by a fungal biopesticide with relatively late mortality initiation, which then kills at a very high rate (Figure 5).

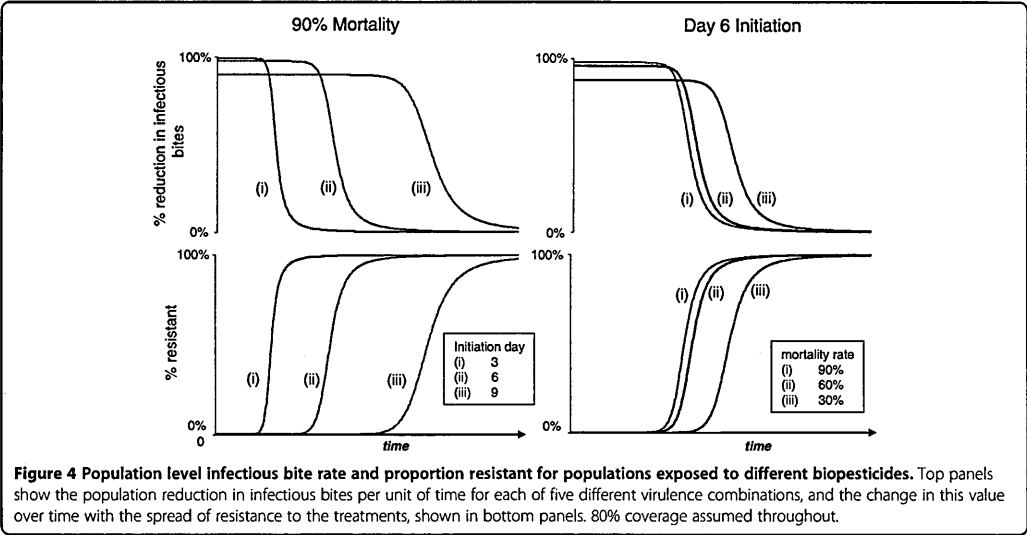
Clearly, the probability that a mosquito contacts and is affected by a vector-control treatment has a significant impact on both the reduction in EIR and reproductive success. Reductions in EIR improve as coverage is increased, but the strength of selection for resistance also increases (Figure 6, left panels). This illustrates the predictable trade-off between the best transmission control, obtained at high coverage, and the best resistance management, obtained at low coverage. When compared to the currently available alternative, a conventional instant-kill chemical insecticide, however, the relative values for EIR reduction and resistance management with the biopesticides are maximized at the high coverage values which correspond to the best transmission control and the strongest selection pressures for



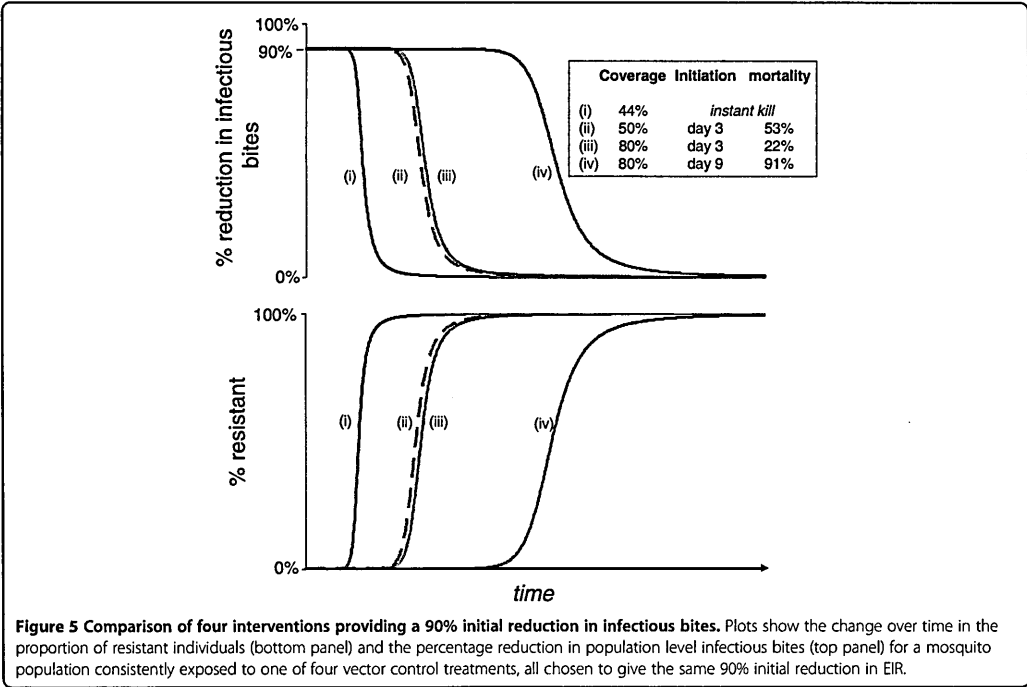
**Figure 2** Illustrative survival curves for a range of simple virulence mortality assumptions. Survival curves illustrating mortalities defined by two simple virulence parameters. For each illustrated pair of values, mortality is zero until the specified initiation day, and is thereafter maintained at the indicated fixed daily mortality rate. Initiation day and mortality rate are the two parameters used to define the assumed incremental mortality generated by a given biopesticide infection.

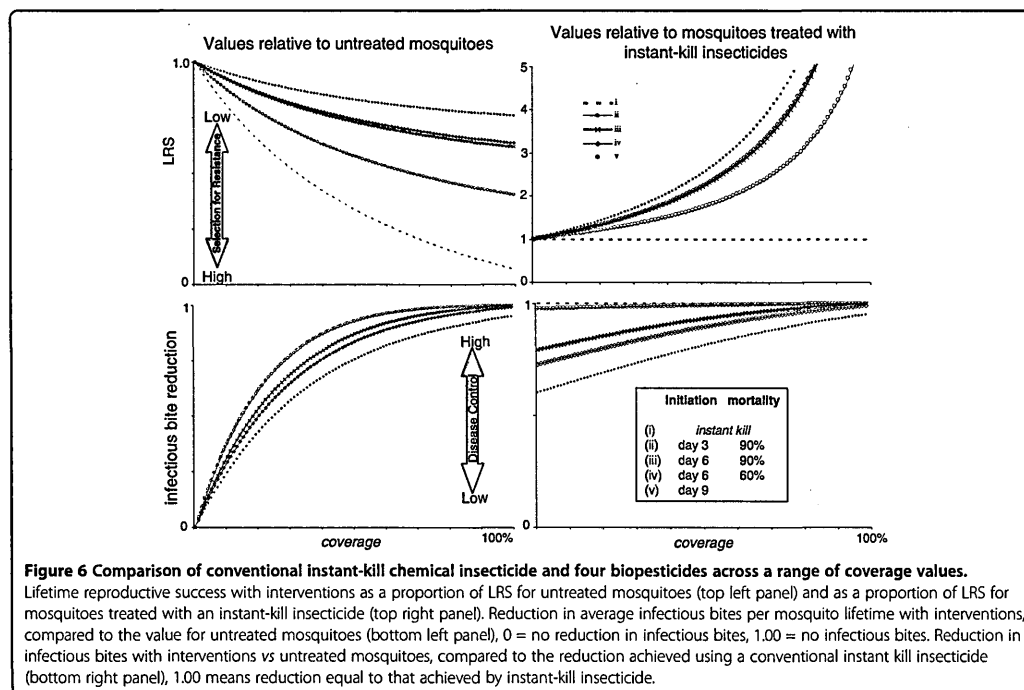


**Figure 3** Comparison of virulence characteristics and fitness costs associated with given reductions in EIR. Top panels show different combinations of values for initiation day (x-axis) and daily mortality rate (y-axis) which achieve the denoted reductions in EIR (RAIB). The mortality rate required to achieve a given RAIB increases for later initiation days, up to an initiation day beyond which the target RAIB cannot be achieved, at which point the plots stop. With 50% biopesticide coverage, no virulence parameter combinations achieve 99% RAIB. Bottom panels show the selection coefficients corresponding to the same set of virulence parameter values, eg, the 99% RAIB value plotted for initiation day 2 gives the fitness cost for susceptibility to a biopesticide with initiation day 2 combined with the mortality rate required to achieve a 99% RAIB. Higher selection coefficients indicate stronger selection pressure for resistance.



resistance (Figure 6, right panels). Even a biopesticide with sufficiently high virulence to match the initial EIR reduction of instant-kill insecticides at the same coverage levels offers some benefit in terms of useful life (Figure 7). This is because fungus-infected mosquitoes are still able to achieve some reproduction before being killed, thus somewhat reducing the selection for resistance.





### Repellency

One of the most commonly used classes of conventional insecticides, pyrethroids, have high contact irritancy (also called excito-repellency), causing approximately 50% of mosquitoes contacting treated surfaces to be repelled without acquiring a harmful dose [23-25,38]. There is no indication of any repellency effects for the fungal biopesticides [26]. For IRS, if 50% of mosquitoes contacting the instant-kill insecticide are unaffected by it, then, for equivalent spray coverage, fungal biopesticides offer better reductions in EIR at all coverage levels, whilst maintaining selection benefits for all but the most virulent strain at the lowest coverage (Figure 8).

### Malaria interactions

Some fungal strains have been shown to have higher virulence in malaria-infected mosquitoes than in those without malaria infection [15]. The trade-off between reducing EIR and resistance management is greatly reduced where fungal virulence is lower in malaria-free mosquitoes, with selection for resistance virtually eliminated if the fungus induces mortality exclusively in malaria-infected mosquitoes (Figure 9).

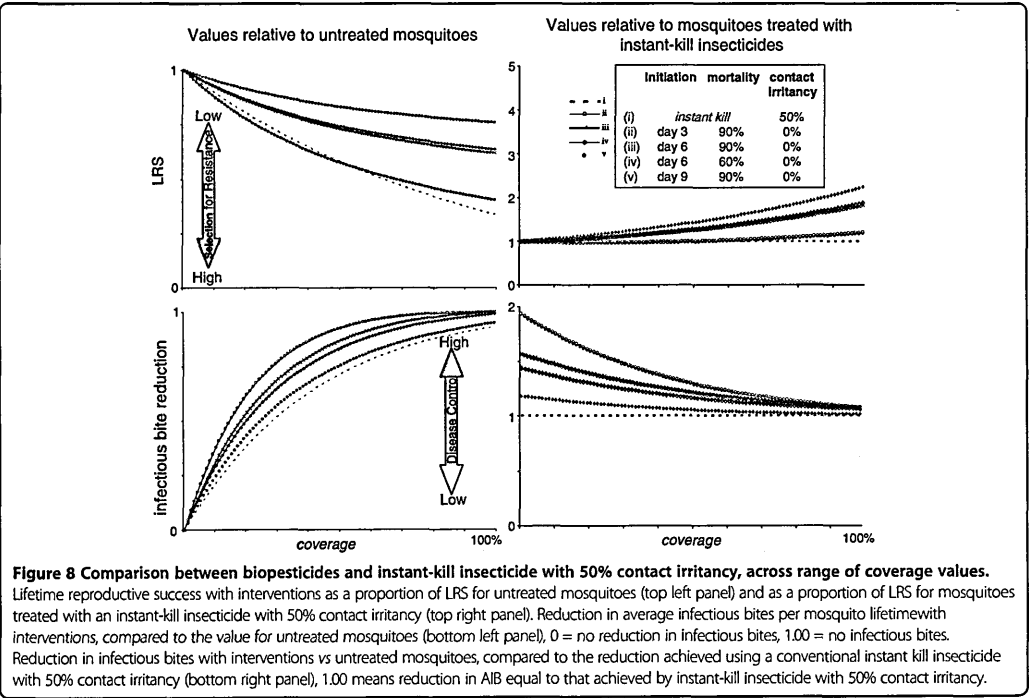
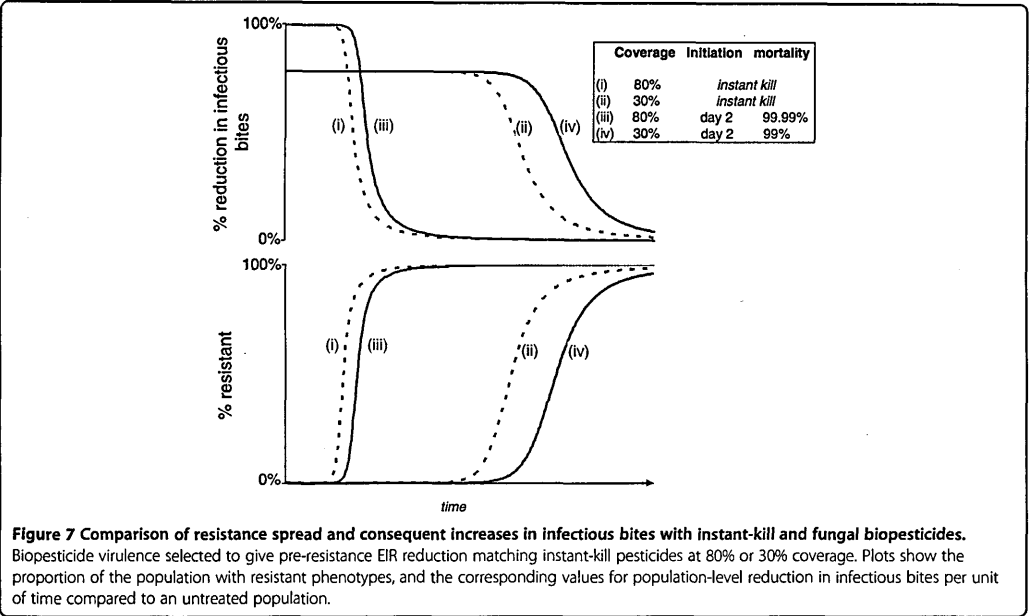
### Discussion

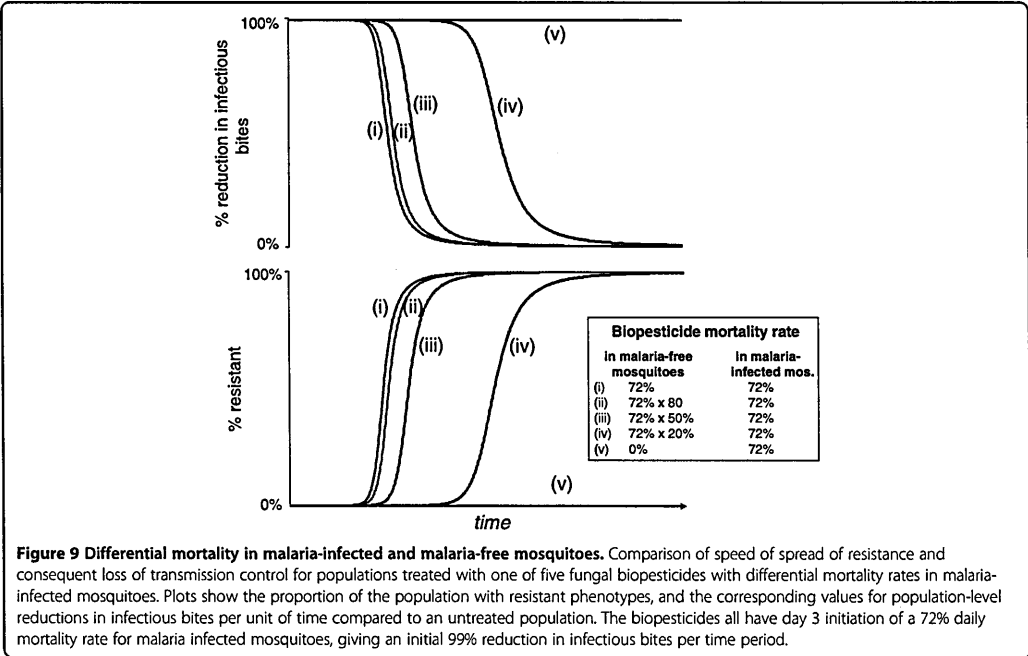
Variation in the virulence characteristics of potential biopesticides offers scope for selecting strains targeted to

provide desirable combinations of reduced transmission and resistance management. A number of virulence phenotypes can provide equivalent levels of EIR reduction (Figure 3), and in general high biopesticide-induced mortality rates commencing as late as possible offer better resistance management for a given level of EIR reduction (Figures 3 and 5). There is nonetheless a trade-off between extending the time taken for resistance evolution to undermine efficacy of a pesticide, and the initial reductions in transmission (Figure 4). In general terms, more virulent fungal strains better reduce transmission initially, but at the cost of stronger selection for resistance, and consequently a shorter useful life (Figure 10).

Although high coverage offers scope to use less virulent fungal strains to reduce EIR, for given virulence parameters, higher levels of coverage also generate stronger selection for resistance, for both conventional and biopesticide interventions. Remembering that the biopesticides must be considered in relation to the best currently used approaches, it is interesting to note that in relative terms, the benefits of biopesticides *versus* conventional instant-kill insecticides are maximized at high coverage for both transmission control and resistance management (Figure 6).

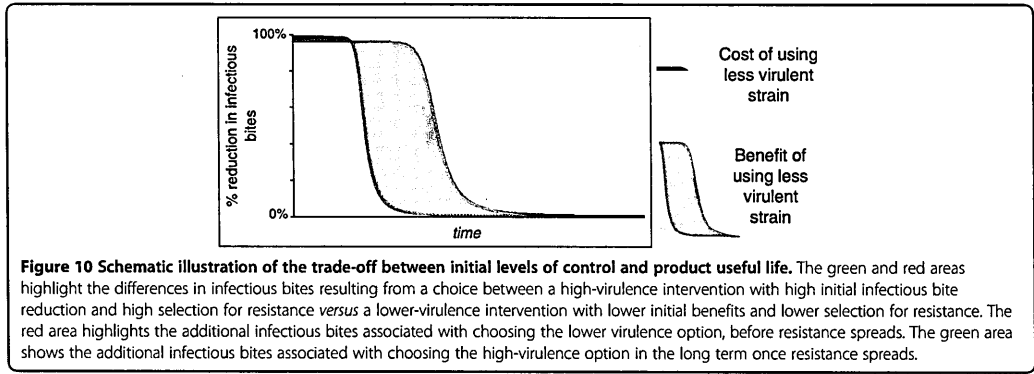
The relative importance of initial control *versus* product lifespan depends on a large number of factors,





including the availability of alternative replacement treatments, the meaning in terms of human morbidity and mortality of a smaller reduction in EIR at the outset, and the realities of public budgets and other resources. The relative costs and benefits also change if the biopesticide is being considered for use as part of a combination treatment with other interventions [34,39,40]. There is, therefore, no simple mathematical optimum for the many possible virulence schedules; the many possibilities need to be considered in context. In so far as it can be done without compromising transmission

control, however, it is clearly beneficial to choose the biopesticide that generates the lowest selection for resistance in a particular context. For resistance management, the aim should be to achieve high levels of coverage, allowing less virulent fungal strains to achieve a given level of control, and maximizing their resistance management benefits over instant-kill insecticides. Even strains sufficiently virulent to match the transmission-reducing characteristics of conventional instant-kill chemical insecticides at matching coverage levels still offer a small benefit in terms of the rate of spread of resistance (Figure 7).



Such a resistance management gain would be enhanced by any fitness costs associated with resistance [8].

The conclusions presented here are independent of the method of resistance (e.g., metabolic or behavioural), provided resistance is genetically determined. It is assumed however that resistance is a binary quality, with mosquitoes either experiencing the full effects of a control measure, or remaining completely unaffected by it. The analysis of the speed of spread of resistance here thus assumes that susceptible mosquitoes experience infections with the specified virulence characteristics, and that resistant mosquitoes have no fungal mortality. In reality, it is more probable that a resistance/tolerance process would operate, with resistant mosquitoes still becoming infected, but experiencing a lower mortality rate than fully susceptible individuals. The spread of resistance would therefore effectively comprise a reduction in fungal virulence, rather than a complete loss of control. Considering the results presented in Figure 4, for example, this would mean that the spread of resistance to the highest virulence biopesticides, rather than comprising a steep function to complete resistance and total loss of transmission control, would move to the curves calculated for sequentially less virulent strains, as resistance converts high virulence strains to low virulence strains, offering even more beneficial resistance management possibilities. Future analyses could explore the impact of hypothetical resistance mechanisms that might operate with respect to conventional and fungal pesticides. The analyses presented here could also be extended to evaluate the impact of malaria infection on mosquito survival, fecundity and behaviour and variation in fecundity with mosquito age.

Certain widely used pyrethroid insecticides have high contact repellency, with studies suggesting that around 50% of mosquitoes landing on treated surfaces may leave before acquiring a fatal dose [23-25,38]. Whilst this potentially enhances the impact of pyrethroid-treated bed nets on transmission by deflecting mosquitoes away from protected humans before they bite, for IRS it results in mosquitoes surviving to potentially transmit malaria in later feeding cycles [24]. Thus, for this group of conventional insecticides, the composite 'coverage' value at a given level of spray cover, would be half that for biopesticides, and could never be greater than 50%. Comparing biopesticide performance with that of a conventional insecticide, and assuming 50% contact repellency (Figure 8) across a full range of coverage values, fungi better reduce transmission than pyrethroid IRS, while still maintaining some resistance management benefits. This suggests that, for all spray coverage values, suitably virulent fungal strains might provide a better option for IRS-based vector interventions than contact-repellent pyrethroids. If only low levels of spray coverage

are achievable, replacing repellent pyrethroids with high-virulence fungal treatments could significantly improve the achievable EIR reduction, without significantly increasing selection for resistance, which is in any case relatively weak at low coverage (Figure 8). Where high spray coverage is achievable, replacing pyrethroids with relatively low-virulence fungal treatments could give improvements in both transmission control and resistance management, since the relative fitness of susceptible mosquitoes would be potentially doubled.

The analysis shows that in all cases, having higher fungal-induced mortality in malaria-infected mosquitoes than in uninfected mosquitoes minimizes the fitness costs associated with a given reduction in transmission (Figure 9). The ideal biopesticide from the resistance management perspective would be one that had little or no impact on mosquitoes not infected with malaria, but was strongly virulent in malaria-infected individuals. This might be possible since malaria infection can impose significant metabolic and immunological challenges to mosquitoes [41-44]. There is only a minimal trade-off between transmission control and resistance management in malaria-linked incremental biopesticide mortality. By changing the fitness cost to the mosquito of malaria infection, pesticides working in this way might also exert selection in favour of vector resistance to malaria, further enhancing the transmission-control benefits from the intervention. Strain selection or genetic modification should ideally target this trait. A further development of this principle would be fungal strains which specifically block development of the malaria parasite in the mosquito, or simply act as a delivery mechanism for anti-malaria interventions in the mosquito host ('para-transgenesis' [10,37]), with minimum survival or fecundity costs to the mosquito. It must be noted, however, that this potentially moves selection for resistance from the mosquito to the malaria parasite, which has so far proved extraordinarily adept at evolving its way out of trouble.

## Conclusions

This analysis shows that fungal biopesticides have the potential to significantly reduce EIR while imposing only weak selection for resistance. There is always a trade-off between the magnitude of the initial reductions in transmission and maintaining those reductions in the longer term. Given the severe human and economic consequences of malaria transmission, choosing an intervention which does not maximally reduce transmission at the outset requires very careful justification. However, the analyses presented here show that fungal biopesticides can offer equivalent or better reductions in transmission than existing interventions in both the short and long term. This is especially true where existing



conventional chemical pesticides have high contact irritancy or resistance to them has already begun to spread. The theoretical analyses presented here should help define the vector mortality profiles required to maximize the sustained malaria control potential of fungal biopesticides, or indeed other novel biological or chemical insecticides.

## Additional files

**Additional file 1: Appendix A.** Derivation of the feeding cycle model. Detailed description of variables and calculations used in the feeding cycle model.

**Additional file 2: Appendix B.** Derivation of the population model. Detailed description of variables and calculations used in the population model.

## Abbreviations

AIB: Average number of infectious bites per mosquito per lifetime; EIR: Entomological inoculation rate, the number of infectious bites per person per unit of time; FCM: Feeding cycle model; IRS: Indoor residual spraying; LLA: Late-life acting; LRS: Lifetime reproductive success; PM: Population model; RAIB: Proportionate reduction in average number of infectious bites per mosquito per lifetime, compared to the number for untreated susceptible mosquitoes.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

PL developed and applied the model and contributed to study inception, interpretation of results and drafting of the manuscript. UG reviewed the model and contributed to drafting of the manuscript. MT and AR contributed to study inception, interpretation of results and drafting of the manuscript. All authors read and approved the final manuscript.

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